REPORTS

OF THE

COMMISSION

APPOINTED BY

THE ADMIRALTY, THE WAR OFFICE, AND
THE CIVIL GOVERNMENT OF MALTA,

FOR THE INVESTIGATION OF

MEDITERRANEAN FEVER,

UNDER THE SUPERVISION OF AN

ADVISORY COMMITTEE

OF

THE ROYAL SOCIETY.

PART V.

LONDON:
HARRISON AND SONS, ST. MARTIN'S LANE,
PRINTERS IN ORDINARY TO HIS MAJESTY.

Price Two Shillings and Sixpence.

FEBRUARY, 1907.

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I. EXPERIMENTS WITH MONKEYS ON VARIOUS POSSIBLE PATHS OF INFECTION IN MEDITERRANEAN FEVER.

By Staff-Surgeon E. A. Shaw, M.B. Cantab., Royal Navy.

The major portion of the following experiments were undertaken pursuant to suggestions made by the Advisory Sub-Committee and received by me in December, 1904. As some of them are merely repetitions of experiments already reported by members of the Commission, it is hardly necessary to describe these with great wealth of detail.

I. With Dust Infected with *Micrococcus melitensis* Derived from Spleen Cultures.

The dust was prepared by grinding up and sterilising yellow Sliema soil, a layer of which, 3/16 of an inch deep, was saturated with the emulsion obtained from one agar slope of a seven-day growth of *Micrococcus melitensis*, second generation from human spleen. This was then dried in incubator and plated to determine presence of living *Micrococcus melitensis*; then carefully powdered and used as described.

(a) *Monkey Placed in Air-tight Box and Artificial Dust Storm Created.*

The infected dust was placed at the bottom of a filtration flask which was fitted with a rubber cork through which passed a glass tube, one end of which almost touched the bottom, the other end being connected by rubber tubing with the nozzle of a pair of bellows; a side tube from the upper portion of the flask was fitted with another piece of rubber tubing which was passed into the interior of an air-tight tin box with one glass side, the orifice of entry for the rubber tube being luted with cotton wool. The monkey was enclosed in a bag with a draw-string round its neck and placed with its face in direction of the dust, which issued on gently using the bellows, which were kept going for 20 minutes, the monkey allowed to remain 10 minutes longer, then removed and everything disinfected.

Monkeys No. 64, in December, and No. 48, January, 1904–5, were each subjected to this procedure once and once only, the intention being to obtain definite information as to their incubation period for this form of infection should the experiment succeed. Both monkeys had been under careful observation prior to the experiment and neither had ever given the slightest indication of fever, either by rises of temperature or in response to frequently repeated serum agglutination tests.

Result.—Neither monkey became infected.

(b) *Infected Dust Blown up Monkeys' Nostrils.*

A piece of glass tube of about 1/8 of an inch diameter was taken, one end made conical and rounded off in the Bunsen flame, about half a gram of infected dust placed in it, the larger end connected by a rubber tube lightly plugged with cotton wool to the bulb of an enema. The monkey was held by an assistant, the conical end of the dust-containing glass tube placed in one nostril and pressed in till an air-tight junction was effected between the two, then compression of the rubber enema bulb puffed the infected dust into the nasal cavity. The plug of cotton wool above mentioned functioned as a non-return valve and prevented infected dust regurgitating into the enema bulb. The presence of living *Micrococcus melitensis* in the dust was proved by plating it out each time it was used.

Monkeys Nos. 65, 77, and 103 were subjected to this procedure.

Monkey No. 65.—On December 29, 1904, was so treated. Prior to this its temperature was being taken and the agglutination reaction examined for frequently; this was first obtained in a dilution of 1 in 20, January 14; in a dilution of 1 in 40, February 16; in a dilution of 1 in 400, February 18. Its first wave of temperature did not begin till February 8, and only lasted till the 14th, inclusive, that is seven days; this would give an incubation period up to the first rise of temperature of 40 days. On February 14, blood was taken aseptically from the saphenous vein of right leg (the manner of doing this will be described under "immunity" experiments); incubated in broth for seven days, then sub-inoculated on to agar slopes, whence *Micrococcus melitensis* was recovered and verified three days later. This animal was later used for immunity experiments.

Monkey No. 77.—Arrived from Calcutta, March 3, 1905. Dust prepared as above described and blown up nostrils April 26, 1905. This monkey never presented any agglutination reaction or fever. It died of diarrhœa June 9. The customary *post-mortem* inoculation of plates and tubes from organs, etc., were made. No *Micrococcus melitensis* was recovered.

Monkey No. 103.—Arrived from Calcutta, March 3. Dust prepared as above described and blown up nostrils once a day for five days, from June 29 to July 3, inclusive; the reason for repeating this procedure five times was that this monkey had a trick of shutting off its nasal cavity from its pharynx, so as to make a closed chamber of the former which greatly interfered with, practically prevented in fact, the puffing of the dust into its nostrils, so that a satisfactory dose was not administered till the fifth day.

The agglutination reaction was first obtained August 3, 1 in 30, was 1 in 160 August 6, and 1 in 800 August 10, and 1 in 2,000 August 20. It presented no distinct wave of fever till August 10, when its evening

temperature went up to 103°, remaining at or above this till the 19th, when it gradually descended; this would give an incubation period from July 3 to August 10 of 38 days. Blood was taken from vein of leg, as in Monkey No. 65, on August 11, put in broth and incubated. *Micrococcus melitensis* was recovered and verified from this on August 23. During September this monkey suffered with bad diarrhœa, dying on September 30. The usual inoculations of plates and tubes made *post-mortem* failed to yield any *Micrococcus melitensis*.

Result.—Two monkeys out of three thus experimented on became infected, the infection being absolutely proved by the recovery of *Micrococcus melitensis* from their blood during life, the incubation periods being respectively 40 and 38 days.

(c) *Infected Dust Placed in Conjunctival Sacs.*

Four monkeys experimented with, Nos. 76 and 78 old series, and 28 and 29 new series. In each before experimentation the eyes were tested with a solution of fluorescin and carefully examined for conjunctival scratches and abrasions; none were found. The monkey being held by two assistants, with one's left fingers the two eyelids were separated, and with the right hand a little of the infected dust was dropped from a small spatula into the conjunctival sac, the eyelids being kept apart until the dust was thoroughly saturated with tears, and then the animal was released.

Monkey No. 76.—Dust dropped into eyes January 23, 1905; the animal died of diarrhœa February 7; there had been no agglutination reaction or rise of temperature during this period. A very large number of plates and tubes were inoculated from the various organs *post-mortem*, but no *Micrococcus melitensis* was recovered.

Monkey No. 78.—Infected dust dropped into eyes on May 6, 1905; May 31, 1905; June 29, 1905; August 6, 1905; August 26, 1905. This animal's serum was examined twice a week for agglutination reaction up till October 15, invariably with a negative result; its temperature was recorded twice daily during the whole period, but there was never any rise of temperature.

Monkey No. 28.—Infected dust dropped into eyes daily for a week commencing October 29, 1905. Agglutination was first found November 19, 1 in 30, and was 1 in 600 November 26. A wave of temperature commenced November 8, with 104° F. (I should put the incubation period here 4 to 10 days), and lasted till the animal's death on November 28. Blood had been taken from its right saphenous vein the day before, November 27, and incubated in broth. *Micrococcus melitensis* was recovered and verified from this on December 7. At the *post-mortem* there was found purulent peritonitis of pelvis and lower part of abdomen. The usual inoculations were made, and on December 1 *Micrococcus melitensis* was found and verified in innumer-

able colonies in the axillary and femoral lymphatic gland plates, and also in the spleen; there were many fewer *Micrococcus melitensis* colonies in the liver plates and fewest of all in the kidney plates.

Monkey No. 29.—Infected dust dropped into eyes daily for a week commencing October 29, 1905. Agglutination reaction first present on November 12, 1 in 30, was 1 in 400 November 19, and 1 in 1500 on November 26, rising to 1 in 2000 on December 10, after which it fell away, being 1 in 1000 on December 24, 1 in 800 January 14, 1906, and 1 in 400 on January 28. The first wave of temperature began November 20 and lasted till November 28, thus giving an incubation period of 16 to 22 days. Blood was taken from right saphenous vein on November 21, incubated in broth and sub-cultured on agar. *Micrococcus melitensis* was thus recovered and verified November 30. The animal was placed in a cage with an uninfected monkey (131), separated from it by wire netting as a limited contact experiment, on November 22. It developed acute diarrhœa on January 28 and died on the 30th. The usual *post-mortem* inoculations were made from organs and lymphatic glands and *Micrococcus melitensis* was recovered, four colonies only in one plate from a left axillary lymphatic gland.

Result.—Monkeys Nos. 76 and 78 negative. Monkey No. 28 was infected, incubation period 4 to 10 days. Monkey No. 29 was infected, incubation period 16 to 22 days.

II. With Dust first Saturated with Mediterranean Fever Patient's Urine, Naturally Containing *Micrococcus melitensis*, then Dried and Blown up Monkeys' Nostrils.

Four monkeys thus treated. The urine made use of was that from ambulatory Cases 9 and 11 (see Vol. IV of these reports). These two urines have been collected and plated out continuously twice weekly since August, 1905, and have never failed to contain living *Micrococcus melitensis* up to time of writing (end of April). Small quantities of sterile dust were placed in watch glasses, saturated with the urine and dried in the incubator, and this was blown up the monkeys' nostrils in the manner already described (Section I (b)).

Monkey No. 104.—Received from Genoa, July 12, 1905. Kept under observation and blood examined for agglutination reaction.

July 25. Dust prepared as described and blown up monkey's nostrils; this was repeated daily till August 27.

August 28 to October 3. Urine dust not administered.

October 4 to November 5. Urine dust daily administered.

November 6. Very seedy.

November 7. Obviously dying. Chloroform given.

Post-mortem made, usual inoculations from all organs and lymphatic glands. No *Micrococcus melitensis* was recovered. During the whole period of the experiment the blood was examined twice a week for

agglutination reaction which never appeared, nor was there any wave of temperature.

Monkey No. 106.—Received from Genoa, July 12. Treated in precisely the same manner as Monkey No. 104, commencing July 25 and repeated daily till the 29th. Unfortunately the animal developed violent diarrhœa and died on July 31. The usual *post-mortem* inoculations from organs and lymphatic glands were made but no *Micrococcus melitensis* was recovered. There was never any agglutination reaction or fever.

Monkey No. 25.—(New Series). Received from Calcutta, October 18, 1905. From October 29 to November 24 Mediterranean Fever urine dust was blown up nostrils daily, then intermitted because of development of diarrhœa.

November 28, 1905, to March 13, 1906, Mediterranean Fever urine dust blown up nostrils daily between these two dates, and then suspended.

During the whole of this time, October to March, periodical examinations for agglutination reaction were being made (once a week) and the temperature was being twice daily recorded. There was never any agglutination reaction present and there was never any fever.

Monkey No. 35.—Received from Calcutta, October 18, 1905.

November 8 to November 24 urine dust blown up nostrils daily.

November 25 to November 28. This suspended because of diarrhœa.

November 29 to December 24. Urine dust blown up nostrils daily, then again suspended five days.

December 29 to March 4. Urine dust blown up nostrils daily.

March 5 again suspended. Monkey seedy.

March 11. Has developed acute diarrhœa.

March 13. Died.

As with No. 25, agglutination reaction had been examined for weekly during the whole period but had never been present. Temperature had been recorded twice daily but there had never been any fever. The usual *post-mortem* inoculations of plates and tubes from organs and lymphatic glands were made immediately after death, incubated and carefully examined for *Micrococcus melitensis*, but none was recovered.

Addendum.—Monkey No. 25 died, without obvious cause or discoverable *post-mortem* lesion, on April 5, 1906. It was very thin and emaciated. The usual *post-mortem* inoculations from all organs and lymphatic glands were made, but no *Micrococcus melitensis* was recovered.

Result.—Thus of four monkeys experimented with as described, none became infected from dust contaminated with urine naturally containing *Micrococcus melitensis*.

III. Contact Infection.

(a) LIMITED CONTACT *in mosquito-proof hut between an Infected and an Uninfected Monkey living in same box on a bottom of wire netting and separated by a vertical wire netting partition, and Possible Infection by Urine and Mosquitoes Excluded, Infection by Skin and Skin Parasites possible.*

Monkey No. 81.—Was kept under observation eight days for temperature and agglutination reaction, and then on May 30, 1905, placed as above with Monkey No. 79 which, on May 15, had received a subcutaneous injection of living *Micrococcus melitensis* and which on May 30 presented an agglutination reaction of 1 in 800 and was in a wave of fever. Monkey No. 79 died on June 4, and *Micrococcus melitensis* was recovered *post-mortem* from its spleen. In order to ascertain approximately incubation period, should infection occur, Monkey No. 81 was left to live alone in his box, under observation for fever and agglutination reaction. Neither having presented themselves by July 20, on this date, Monkey No. 80 (whose blood had been proved by culture in broth to have contained *Micrococcus melitensis* on July 11) was placed in the compartment formerly occupied by Monkey No 79. On August 6, Monkey No. 81 developed diarrhœa, which in spite of treatment got worse, this monkey dying on August 19. The usual *post-mortem* inoculations from organs and lymphatic glands were made and incubated, but no *Micrococcus melitensis* was recovered; both temperature and blood were under continuous observation, but there was never any fever or agglutination reaction.

Monkey No. 131.—A normal, uninfected animal was, on August 29, placed in one compartment of same box (which had previously been thoroughly disinfected) with in the other compartment Monkey No. 118, a milk *Micrococcus melitensis* infected monkey, whose blood had been demonstrated by culture in broth to have contained *Micrococcus melitensis* on August 12, and which was in a wave of fever and presented an agglutination reaction of 1 in 1000 on August 27. The two monkeys remained in same box till October 18, when the infected one (No. 118) died of acute diarrhœa, *Micrococcus melitensis* being recovered from one femoral gland *post-mortem*. Monkey No. 131 now remained alone till November 22, when Monkey No. 29 (new series) was placed in the compartment vacated by Monkey No. 118; Monkey No. 29 had been infected by placing dust containing *Micrococcus melitensis* into its eyes, and *Micrococcus melitensis* had been demonstrated to have been present in its blood on November 21 by culture in broth. These two monkeys remained together till January 3, 1906, when Monkey No. 131, which had previously developed diarrhœa, died. The usual *post-mortem* inoculations of plates and tubes from organs and glands were made and incubated, but

Micrococcus melitensis was not recovered; the animal had been under continuous observation for fever and agglutination reaction, but never presented either. Its companion (Monkey No. 29) died January 30, 1906, and *Micrococcus melitensis* was recovered *post-mortem*.

(b) FULL CONTACT *between an Infected and an Uninfected Monkey living in same box, Mosquito Infection alone Excluded.*

Monkeys Nos. 82 and 115 were the uninfected monkeys thus experimented on.

Monkey No. 82.—Kept under observation for eight days for temperature and agglutination reaction, then placed, on May 30, 1905, with Monkey No. 80, which, on May 15, had received a subcutaneous injection of *Micrococcus melitensis* and on May 29 was in a wave of fever and presented an agglutination reaction of 1 in 3000, *Micrococcus melitensis* being recovered from its blood in July, 1905.

Monkey No. 82, on July 9, first presented agglutination reaction in a dilution of 1 in 40, which had risen to 1 in 1000 by July 23, but it never presented any distinct wave of fever, its temperature never rising above 102°·6 F. On July 15 blood was taken aseptically from the right saphenous vein of Monkey No. 82, incubated in broth, and on July 23 *Micrococcus melitensis* was recovered and verified from this. On August 2 this animal was lent to Major Horrocks to be used for mosquito-infection experiments at Lazzaretto. In October its agglutination had fallen to 1 in 80 and to 1 in 20 in November.

Monkey No. 115.—Arrived from Genoa, July 12, 1905. Kept under observation for agglutination reaction and temperature till July 20, when it was placed in same cage, in full contact under mosquito-proof conditions, with Monkey No. 100, which on June 28 had received a drop of emulsion of *Micrococcus melitensis* in the eye, and whose blood on July 18 had been proved to contain *Micrococcus melitensis*, and which on July 20 was in a wave of fever and presented an agglutination reaction of 1 in 1500. These two animals lived together till November 1, when Monkey No. 115 never having presented the slightest indication of infection, Monkey No. 100 was taken out of the box and replaced by Monkey No. 27 (new series) which was just commencing a wave of fever, having received a subcutaneous injection of *Micrococcus melitensis* on October 25 and whose blood was demonstrated by broth culture to have contained living *Micrococcus melitensis* on November 1. Monkeys No. 115 and 27 lived together till the death of the latter on March 2.

Monkey No. 115 had never presented any wave of fever or agglutination reaction from July 20, 1905, to March 20, 1906, during which period its temperature had been recorded twice daily and its blood examined for agglutination reaction to *Micrococcus melitensis* once weekly.

Result.—Limited contact experiments on two monkeys, Nos. 81 and 131 ; neither infected.

Full contact experiments on two monkeys; Monkey No. 82 was infected as evidenced by recovery of *Micrococcus melitensis* from its blood during life, incubation period impossible to determine. Monkey No. 115 was not infected. I consider Monkey No. 82 as almost certainly infected through the urine of Monkey No. 80. In the winter of 1904–05 I caused partitions to be placed separating and isolating the boxes for monkeys on the terrace, a plan of which is given in Vol. I of these reports. The floor of the terrace is concrete cement, and so each partition was embedded in cement at its junction with the floor. The object of this was to eliminate the possibility of accidental infection through urine. So far as I know not a single monkey has been infected save as the result of purposeful experiment since this was done.

IV. Infection through various Mucous Membranes with Emulsion of *Micrococcus melitensis* derived from Human Spleen Growths and Dropped into

(a) *Conjunctival Sac.*

In these experiments, to ascertain as far as possible that one was attempting to infect through an unbroken mucous membrane, and not through a scratch in it, a solution of fluorescin was first applied in the manner familiar in ophthalmic practice, and the mucous membrane carefully examined with a lens for staining of abrasions or scratches.

Two monkeys, 61A and 100 were both thus experimented on.

Monkey No. 61A—After being under preliminary observation to determine its freedom from infection, on December 19, 1904, it received in each conjunctival sac one drop of an emulsion in distilled sterilised water of a seven-day growth of second generation of *Micrococcus melitensis* from human spleen. Its temperature remained practically normal till the morning of January 3, when it was 103°, rising to 104° same evening, and being 106° on morning of January 4, returning, after describing a characteristic wave, to the normal on January 13. Agglutination reaction was first found on January 3 in a dilution of 1 in 40, on January 9 was 1 in 160, on January 16 1 in 1500. On January 14, blood was taken aseptically from right saphenous vein and incubated in broth; *Micrococcus melitensis* was recovered from this and verified in the usual way. The animal was later used for an immunity experiment. The incubation period here is obviously 15 days.

Monkey No. 100.—After preliminary observation, to determine freedom from infection, this animal received on June 28, 1905, into each conjunctival sac one drop of an emulsion of *Micrococcus melitensis*

derived from human spleen. On July 13 the agglutination reaction with *Micrococcus melitensis* was first obtained. On July 12 it commenced a wave of fever ranging about 104°, being therefore not so marked as in Monkey No. 61A. On July 16, blood was taken aseptically from its right external saphenous vein and incubated in broth; from this living *Micrococcus melitensis* was later recovered and verified. The animal was later also made use of for an immunity experiment. The incubation period here was 14 days.

Result.—Both monkeys experimented on became infected, the infection being absolutely proved by the recovery of *Micrococcus melitensis* from their blood during life, the incubation periods being respectively 15 and 14 days.

(b) *Emulsion of* Micrococcus melitensis *Dropped in Trachea.*

This was Experiment 22 suggested by Advisory Sub-Committee and allotted to me for performance. It was at first intended to use a small serum syringe with which to deposit the single drop of emulsion in the trachea, but on rehearsing the experiment so much uncertainty was felt owing to the inability to see the end of the column of fluid in the needle, and from the absence of a stop on the needle the risk of puncturing the opposite side of the trachea seemed so great, as to render it extremely likely that the primary condition of the experiment, which was that there should be no soiling of any wound surface with *Micrococcus melitensis*, would not be realised; the object being to ascertain possibility of infection and duration of incubation period in the case of unbroken tracheal mucous membrane. The method ultimately decided on was the following:—A sterile glass pipette was taken, and its fine terminal portion bent in the flame at a right angle about $1\frac{1}{2}$ inches from its point; the butt end of the pipette had been as usual lightly plugged with cotton wool and was now fitted with a piece of rubber tube provided with a mouth-piece; by suction the equivalent of two drops of sterile normal salt solution was drawn into the capillary portion of the pipette, then a bubble of air, and then the equivalent of one drop of emulsion of *Micrococcus melitensis*; thus one had in the capillary portion of the pipette two columns of fluid separated by a bubble of air; that nearest the thick end of the pipette being sterile salt solution, that nearest its terminal extremity, and hence the first to make its exit, being emulsion of *Micrococcus melitensis*, the terminal end of which column was arranged to coincide with the situation of the right angle already mentioned; these two columns were maintained motionless in the long capillary part of the pipette by putting a spring clamp on the rubber tube; and finally the middle of the $1\frac{1}{2}$ inch terminal part of the pipette was placed in the peep light of a Bunsen burner, and drawn out sharply very fine; this left a small shoulder, and the capillary filament was broken $\frac{1}{8}$th of an inch from this with a pair of

sterilised scissors, and thus the terminal fine end of the pipette was sterile. On rehearsing with this on a dead monkey, everything went satisfactorily.

The trachea below the larynx, which in the monkey is enclosed in the fused sternohyoid muscles of both sides, having been laid bare, the sterilised pointed extremity of the prepared pipette was passed between two rings of the trachea, being arrested with its point just inside the trachea by the little shoulder mentioned; the mouth-piece of the rubber tube being in the mouth a little air-pressure was got up and the clamp removed; the column of *Micrococcus melitensis* emulsion at once disappeared from sight, followed by the bubble of air and then by the column of sterile normal saline which was intended to wash away traces of the *Micrococcus melitensis* emulsion from the tip of the pipette and so prevent its soiling the puncture of entry on withdrawal. The experiment as described was performed on Monkey No. 48 (which had been subjected to the usual preliminary observation) under chloroform on January 31, 1905, the animal being placed on a board sloping at about 30° from the vertical so as to allow gravity to act on the fluid injected into the trachea. The operation wound had healed on February 6. It was examined periodically for agglutination reaction, which first appeared on February 16 in a dilution of 1 in 320, rising to 1 in 3000 on February 20. A wave of temperature began on the evening of February 15, when it was 103°, ascending daily till it was 106° on February 20, thus giving an incubation period of 15 days. On February 21 blood was taken aseptically from the right external saphenous vein and incubated in broth. *Micrococcus melitensis* was thus recovered and verified. The animal seemed to fully recover and be quite well during second week in March, but on March 15 was very ill and died on the morning of March 16. The usual *post-mortem* inoculations of plates and tubes from the various organs were made, and *Micrococcus melitensis* was recovered, but from the spleen only. At the *post-mortem* there was no trace of the operation save the scar in the skin; all had healed; there was a good deal of frothy mucus in the trachea, and both lungs were hepatised; inoculations from these yielded a Gram-staining, acid-producing coccus which was not further investigated.

Result.—Monkey No. 48 was infected through the mucous membrane of the trachea, the infection being proved by the recovery of *Micrococcus melitensis* both during life and after death, the incubation period being 15 days, similar to that in the case of the two monkeys infected through the conjunctival mucous membrane.

(c) *Emulsion of* Micrococcus melitensis *Dropped into Nostrils.*

Monkey No. 118 was received from Genoa, July 12, 1905, was kept under observation for temperature and agglutination reaction till

July 20, when, being free from infection, it was held on its back and three drops of an emulsion of *Micrococcus melitensis*, derived from the milk of No. 2 Bighi Goat of July 13, was dropped into each nostril, the position of the monkey's head being such as to allow this to trickle over the turbinate bones. Its blood was frequently examined for agglutination reaction, which first appeared on August 3 in a dilution of 1 in 30, rising to 1 in 320 on August 6 and 1 in 960 on August 10. It commenced a wave of fever on August 3, when its evening temperature ran up to 104°, being on August 4 104°·6, and on August 5 105°, thus giving an incubation period of 14 days. Blood was taken aseptically from its external saphenous vein on August 12, and was incubated in broth. *Micrococcus melitensis* was recovered and verified from this. During September and October the animal suffered intermittently from diarrhœa, ultimately dying on October 18. The usual *post-mortem* inoculations of plates and tubes were made, and *Micrococcus melitensis* was recovered and verified, but only in small quantity, four colonies from one femoral lymphatic gland.

Result.—Monkey No. 118 was infected through the nasal mucous membrane, as proved by the recovery of *Micrococcus melitensis* during life and after death, the incubation period being 14 days, similar to that in the case of infection through the conjunctival and tracheal mucous membranes.

V. Infection by Mosquitoes.

Monkey No. 76, uninfected, had been subjected by Major Horrocks, on September 22, 27, and 29, 1904, to the bites of mosquitoes which had 48 hours previously fed on an infected monkey, after which the animal was turned over to me for continuance. The method I adopted was to place four mosquitoes—all procurable varieties being utilised—in a large pill box with a glass top and a coarse gauze bottom; two sets of six of these boxes were used. The insects were first applied to the hypochondriac regions of the infected monkeys (Monkey No. 60A subcutaneously injected September 16 and Monkey No. 72 food-infected September 19), kept there till at least three of the four in each box had fed, the date and monkey's number noted on the rim of the box, which was then placed in the dark for 48 hours, after which it was brought out and applied to the uninfected monkey till feeding had taken place. The object of having two sets of six boxes was to enable one to apply presumably infected mosquitoes every two days instead of every four. Whenever all the mosquitoes were dead another box was commenced with insects obtained from the Central Civil Hospital ward in which the Mediterranean Fever cases were accommodated. Some of the mosquitoes, generally *Stegomyia fasciata*, would live as long as four weeks, alternately feeding on the uninfected and the infected monkeys, Culex seldom more than four or five days.

The experiment as described was continued by me for just over two months till December 1, when it had to be discontinued owing to the difficulty of obtaining and keeping the mosquitoes alive. Monkey 60A was not used for infecting mosquitoes after the end of September. The infectivity of Monkey No. 72 was kept up by subcutaneous injections of living *Micrococcus melitensis* on October 18 and November 10, and this organism was recovered and verified from its blood on October 10 and 24 and November 6 and 21. The temperature of Monkey No. 76 was recorded twice daily, and its blood examined once weekly, but up to the date of its death on February 7, 1905, it never presented any fever or agglutination reaction, nor was any *Micrococcus melitensis* recovered from its organs *post-mortem*. (It was used for an abortive dust experiment from January 27 to February 7.)

Result.—Negative. Monkey No. 76 remained uninfected.

VI. To Determine Infectivity of Sea Water Fouled with Sewage.

Monkey No. 71, on which Major Horrocks had begun this experiment, was turned over to me at end of September, 1904. Water, often visibly fouled with sewage, was collected for me from the immediate neighbourhood of H.M.S. "Egmont," the stationary depôt ship at Malta which has living on board a number of men varying from 400 to 700. Three bottles full totalling 1,800 c.c. were taken and their contents passed through a Berkefeld filter which had been ascertained by previous experiment* not to pass *Micrococcus melitensis*. The deposit on the filter candle was then washed by passing 600 c.c. of distilled water through, and then with a sterile swab the deposit was emulsified in 10 c.c. of this and with a sterile syringe injected subcutaneously between the shoulders of Monkey No. 71. This I repeated three times a week from September 29 to November 9, by which time the equivalent of 37·8 litres of sewage fouled sea water had been administered. By now this monkey had become very thin and wasted, on November 11 it was decidedly ill and died on the 12th. The usual *post-mortem* inoculations of tubes and plates from viscera, etc., were made, but no *Micrococcus melitensis* was recovered. The temperature and blood had been under continuous observation, but there was never any fever or agglutination reaction.

Result.—Negative. Monkey No. 71 was not infected.

VII. Feeding Experiments.

(a) *Monkeys Fed with Boiled Potatoes Contaminated with Mediterranean Fever Urine naturally containing Living* Micrococcus melitensis.

The urine used was obtained from my Ambulatory Cases IX and XI, and it was demonstrated by plating twice weekly from August, 1905, to

* See p. 108, Part I of these Reports.

April, 1906, to have contained living *Micrococcus melitensis* during the whole period of these experiments. Each monkey was given once a day half a potato on which had been spread about a teaspoonful of infected urine; they invariably ate the whole or a portion of this.

Monkey No. 120 was received from Calcutta on July 28, 1905, was fitted with a leather-covered collar and chain, as were all the monkeys in these feeding experiments, so as to avoid abrasions of the mouth, and their food was invariably soft. After the customary preliminary observations, potatoes, prepared as above, were given to it daily for a period of 29 days commencing on August 7. The animal died on September 26. In my absence Captain Kennedy kindly made the usual *post-mortem* inoculations of tubes and plates from the organs; no *Micrococcus melitensis* was recovered. The animal's temperature had been under observation twice daily and blood twice weekly. There was never any wave of fever or any agglutination reaction.

Monkey No. 120 was not infected.

Monkey No. 121.—Similarly experimented with.

August 7 till September 5, 1905, fed daily with urine-contaminated potatoes.

September 6 till March 11, 1906, fed every Tuesday and Friday without intermission with *Micrococcus melitensis*-containing-urine-contaminated potatoes. This animal had been under close observation the whole period, it had had no wave of fever nor had its blood ever reacted.

Monkey No. 121 had not been infected.

Monkey No. 26 (New Series) was similarly experimented with, commencing October 29, 1905, and continuing twice weekly till March 11, 1906, when the experiment was suspended. This animal similarly had resisted infection thus sought to be conveyed.

(b) *Monkeys Fed with Potatoes Soiled with Dust which had been Saturated with Mediterranean Fever Urine naturally containing* Micrococcus melitensis.

The urine used was from the same patients as in the preceding experiments, and was yielding 200 to 8,000 colonies of *Micrococcus melitensis* per cubic centimetre. The dust was first sterilised, then a teaspoonful, placed in a watch glass, was saturated with a teaspoonful of the urine and left to dry at room temperature for 24 hours; then about half a gramme of it was ground up with half a boiled potato and given to each monkey.

Monkey No. 122.—After the usual preliminary observations feeding was commenced as described on August 7, 1905, and was continued daily for 28 days. From September 6 to February 25, 1906, it was continued twice weekly with an intermission of one week in November and another in December because of diarrhœa, and on March 2, 1906,

the animal died. There had never been any wave of temperature, a very slight agglutination reaction had been noticed on October 4, 1905, then disappearing to appear once again on December 26, and this only in the low dilution of 1 in 30. The usual *post-mortem* inoculations of tubes and plates were made and, much to my surprise, seven colonies of *Micrococcus melitensis* were recovered and verified in a plate prepared from the left kidney; there were none from the other organs or lymphatic glands.

Monkey No. 122 had been infected.

Monkey No. 123.—After the usual preliminary observations to prove freedom from infection, similar feeding was commenced with this animal also on August 7, 1905, and continued daily for 28 days. From September 6 to October 25 it was fed with the Mediterranean Fever urine-dust-contaminated potato twice weekly. On October 21 the agglutination reaction was obtained in a dilution of 1 in 30, rising to 1 in 320 October 25. Blood was taken from the external saphenous vein and incubated in broth, but *Micrococcus melitensis* was not obtained. On October 29 the agglutination reaction was up to 1 in 1500, on the evening of which day its temperature was 103°; there had been no previous indication of a wave of fever, and as next day the temperature was down to 101°·8, fearing I was in danger of missing the irrefutable proof of recovery of *Micrococcus melitensis* from this animal, I administered chloroform, and made the usual inoculations from glands and organs, and after the usual period of incubation thus recovered and verified *Micrococcus melitensis* in numerous colonies from the lymphatic glands and liver, kidney and spleen.

Monkey No. 123 had been infected.

Monkey No. 30.—Feeding was commenced with this monkey on October 31, 1905. Sufficient infected urine-dust was prepared every Tuesday and Friday to allow of giving this monkey a daily portion ground up with potato, and this was continued till December 24; it was suspended from 25th to 29th because of an attaek of diarrhœa which the monkey developed; resumed again December 30 till January 7, 1906, when it was again suspended for the same reason. In spite of treatment the monkey got worse and died January 10. The usual *post-mortem* inoculations of tubes and plates were made, but *Micrococcus melitensis* was not recovered. This monkey had never developed any wave of fever or any agglutination reaction.

Monkey No. 30 had not been infected.

(c) *Monkeys Fed with Potatoes Soiled with an Emulsion of* Micrococcus melitensis *Derived from a Human Spleen Culture.*

A five-day growth of *Micrococcus melitensis*, fifth generation from human spleen, the product of one straight stroke of an infected needle on an agar slope was taken daily, and emulsified in water; a boiled

potato was cut in two, each cut surface was soiled with the emulsion, and the two halves were then given to the two monkeys, Nos. 124 and 125. This was done daily for 29 days, commencing (after the usual preliminary observations) on August 7, 1905. It must be noticed that here one was dealing with enormous numbers of the cocci each time, but at that period it was deemed of considerable importance to determine absolutely whether or not monkeys could be infected by the alimentary canal.

Monkey No. 124.—Feeding commenced August 7. Agglutination reaction first obtained on August 20 in a dilution of 1 in 30, rising to 1 in 320 on August 24, and 1 in 1000 on August 27. There was no very distinct wave of fever, but 103°·2 was recorded on August 20, 104° on the 24th, and 105° on August 28; prior to August 20 the animal's temperature was ranging between 101° and 102°·6. Blood was taken and incubated in broth on August 21, and again on August 29 and September 19. *Micrococcus melitensis* was not recovered from the blood on any of these occasions, so fearing to lose the indubitable proof of actual recovery of the micro-organism, I gave the animal chloroform on October 1, and made the usual *post-mortem* inoculations of plates and tubes. *Micrococcus melitensis* was thus recovered and verified, but only in small quantity :—

7 colonies from the right axillary lymphatic glands
2 „ „ left „ „ „
1 colony „ right femoral „ „
and none from the large viscera or other lymphatic glands.

Monkey No. 125.—Feeding commenced August 7. Agglutination first obtained September 4 in a dilution of 1 in 40, rising to 1 in 500 on September 10, and 1 in 800 on September 28. There was a slight wave of temperature commencing August 25, when it rose to 103°·2, being 104° on the 26th, and remaining at or about this for nine days, when it came down to what it had been before, 101° to 102°·5. Blood was taken and incubated on September 19, but no *Micrococcus melitensis* was recovered, and in consequence chloroform was given on October 2, and the usual *post-mortem* inoculations of slopes and plates made. Here, as in the preceding case, *Micrococcus melitensis* was recovered and verified, but only in small quantity, and only from the lymphatic glands : right femoral 2 colonies, right axillary 1 colony.

Monkeys Nos. 124 and 125 were both infected.

(d) *Monkeys Fed with Potatoes Soiled with Emulsion of* Micrococcus melitensis *Derived from Human Urine.*

This experiment was precisely a repetition of the last, and was carried out in an exactly similar fashion, the only difference being that

a different strain of *Micrococcus melitensis* was used, one derived from the urine of Ambulatory Case No. IX.

Monkey No. 126.—Feeding commenced August 7. Agglutination first appeared on August 24, very slight in a dilution of 1 in 30; it was still 1 in 30, very faint, on August 27, nil on September 1, and 1 in 80 on September 4 and 10. There was no distinct wave of fever, the highest temperatures recorded being 103° on August 28, and 103°·4 on August 29. The animal died on September 14. In my absence the usual inoculations of plates and tubes were made by Captain Kennedy, but no *Micrococcus melitensis* was recovered.

Monkey No. 127.—Feeding commenced as above on August 7, and continued for 29 days. Agglutination first appeared on September 4, being 1 in 120, rising to 1 in 300 on September 6, 1 in 500 on September 10, and 1 in 800 on September 28. The first rise of temperature was on August 23 to 103°·6, and remained between 102°·8 and 104° till September 29. Blood was taken from it on September 14 and incubated in broth, but *Micrococcus melitensis* was not recovered from this, and in consequence I administered chloroform on October 3, and made the usual inoculations of plates and tubes. *Micrococcus melitensis* was thus recovered and verified, but only from the lymphatic glands as follows, none being recovered from the liver, kidneys, or spleen:—

	Micrococcus melitensis colonies.
Left axillary lymphatic glands	103
Right ,, ,, ...	19
Left femoral ,, ...	21
Right ,, ,, ...	4
Cœcal mesenteric ,, ...	3

Monkey No. 126 infection doubtful.
Monkey No. 127 infection proved.

Result.

(*a*) Of three monkeys, Nos. 120, 121, and 26, fed with potato soiled with fresh naturally-infected urine, none became infected.

(*b*) Of three monkeys, Nos. 122, 123, and 30, fed with potatoes soiled with dried dust which had been contaminated with naturally-infected urine, two, Nos. 122 and 123, became infected, No. 30 did not.

(*c*) Of two monkeys, Nos. 124 and 125, fed with potatoes soiled with pure spleen cultures of *Micrococcus melitensis*, both became infected.

(*d*) Of two monkeys fed with potatoes soiled with pure cultures of *Micrococcus melitensis* derived from naturally-infected urine, one, No. 127, became infected, the other, No. 126, doubtful.

The exact incubation period in these food-infected monkeys is not determinable, owing to the impossibility of saying on which particular day infection was received, but that it is very variable is indicated by the varying period from the commencement of the series of feedings to the date of appearance of the agglutination reaction, as follows :—

Serial number of monkey.	Source of *Micrococcus melitensis* infecting the food.	Number of days between commencement of feeding and appearance of agglutination reaction.	Date of commencing feeding.
122	Urine dust	58	August 7, 1905
123	Urine dust	75	August 7, 1905
124	Spleen culture	13	August 7, 1905
125	Spleen culture	28	August 7, 1905
127	Urine culture	28	August 7, 1905

Why fresh naturally-infected urine should have failed to infect, and the same urine dried in dust have succeeded in infecting twice out of three times, is somewhat puzzling to understand, unless in some way, such as by causing minute scratches of the mucous membrane, the dust facilitated the entrance of the micro-organism into the system.

VIII. To Estimate Relative Virulence of Mediterranean Fever Spleen Pulp Direct from Human *Post-mortem* Case.

(Experiment 30, suggested by Sub-Committee.)

Monkey No. 87, after usual preliminary observations, was used for this experiment, and on May 31, 1906, received subcutaneously an injection of about half a gramme of such a spleen emulsified in sterile salt solution. *Micrococcus melitensis* was recovered and verified from this spleen on June 5. Monkey No. 87, up to June 30, 1905, had developed no fever or agglutination reaction. According to previous experience of subcutaneous inoculation of monkeys, when the incubation period is usually three to six days, this meant failure of infection, either due to absence of *Micrococcus melitensis* from the small portion of spleen used, or due to the monkey's powers of resistance. It was decided to try again. On June 30, therefore, another such injection was given to the same monkey, the spleen of this case being also subsequently proved to contain *Micrococcus melitensis*. The agglutination reaction first appeared on July 6, in a dilution of 1 in 80, rising to 1 in 200 on July 16. There was no distinct wave of fever, probably due to the fact that from July 2 to the date of its death the animal suffered severely from diarrhœa, which was absolutely resistant to treatment. The usual *post-mortem* inoculations were made, and

Micrococcus melitensis was recovered (and verified) in profusion from the spleen and mesenteric glands, but not from the bile, liver, or kidneys.

Result.—Monkey 87 was infected with a result somewhat indeterminate as to virulence of such spleen pulp, though the inference would be that it was obviously not great, or the first effort would not have failed, and in the second the micro-organism would certainly have invaded the liver and kidneys as well as the spleen.

IX. To Determine Infectivity of Urine from Ambulatory Cases of Malta Fever.

It was felt to be important to have definite information on this point; and to ensure that such urine did get into the system of the monkey experimented on, I determined to inject it subcutaneously. Accordingly, after the usual preliminary observations, Monkey No. 105 was on July 17 injected between the shoulders with 8 c.c. of fresh urine from Ambulatory Case No. IX (B. Worley, No. 1657). This injection was repeated on the 18th and 19th; a specimen of the urine used was plated each time and subsequently proved to contain living *Micrococcus melitensis.* The animal's blood was examined for agglutination reaction twice daily, and it first appeared on July 31 in a dilution of 1 in 20, rising to 1 in 160 on August 3, 1 in 500 on August 6, and 1 in 1000 on August 10. It commenced a wave of fever on July 31, when its temperature was up to 104°, being 105° on August 5, and running down to nearly normal on August 8. Blood was aseptically taken from the saphenous vein on August 11, and incubated in broth. *Micrococcus melitensis* was thus recovered and verified. From August 3 till its death on September 7, it suffered intermittently from diarrhœa. The urine, which had been injected under the skin of the back between the shoulders, was absorbed, and there was never any abscess formation, or other appearances usual with subcutaneous urine extravasation in the human subject. In my absence, Captain Kennedy kindly made the usual *post-mortem* inoculations of plates and tubes, and *Micrococcus melitensis* was recovered from spleen, liver, and lymphatic glands, not from the kidney or heart's blood.

Result.—The urine of this ambulatory case of fever was thus proved to be infectious.

X. Subcutaneous Inoculation of Monkeys with Pure Cultures of Living *Micrococcus melitensis.*

(Experiments 26, 27, 28, suggested by Sub-Committee.)

This has now been done comparatively frequently, and as the results of this procedure closely resemble each other in each individual

case, a sufficient number of which have already been reported in detail in former volumes of these Reports, I do not propose to do more than refer collectively to my own cases. Suffice it to say, that this procedure invariably results in a very few days in a typical attack of fever, with development of an ultimately high agglutination reaction, presence of *Micrococcus melitensis* in the peripheral blood, etc. The most interesting feature is the comparatively short incubation period as calculated from date of infection to commencement of wave of temperature; this is shown in the accompanying table:—

Serial number of monkey.	Date of inoculation.	Date of appearance of agglutination.		Date of commencing wave of fever.		Incubation period.
78	October 15, 1905	October 21,	$\frac{1}{30}$	October 18,	105°	3
79	May 15, 1905	May 22,	$\frac{1}{1000}$	May 20,	104°·4	5
80	May 15, 1905	May 22,	$\frac{1}{1500}$	May 19,	105°	4
27	October 25, 1905	November 1,	$\frac{1}{30}$	November 1,	103°·4	7
37	February 3, 1906	February 9,	$\frac{1}{30}$	February 6,	105°	3
38	February 3, 1906	February 7,	$\frac{1}{30}$	February 5,	105°	2

It has been usual to examine experimental monkeys for agglutination reaction twice a week, and it is to be presumed, though it was not observed, that this reaction was present in Monkeys Nos. 79 and 80 after May 19, when it was absent, but before May 22 when it was so high.

Remarks.

A consideration of the foregoing experiments leads to the conviction that the monkey, though less tolerant of infection by *Micrococcus melitensis* than any other laboratory animal, reacting for instance with a far more marked wave of fever than the goat, is not an easy animal to infect unless given *Micrococcus melitensis* pure and in relatively large amount. Thus in the dust experiments the two monkeys, Nos. 48 and 64, which took infected dust into their noses by their own inspiration efforts, remained uninfected, while of three monkeys in which it was puffed up their nostrils, two, Nos. 65 and 103, became infected. In the first case the monkeys were noticed to be breathing as quietly as possible, and inspiring as little infected dust as they were able; in the second it was puffed well back into the nasal cavity and was afterwards noticed at the back of the pharynx; this was looked for in Monkeys 48 and 64, but apparently they never took it in in sufficient quantity for it to be thus visible. Of the 10 monkeys used for feeding experiments, four were receiving *Micrococcus melitensis* in very large quantity and in pure culture, and of these, three, Nos. 124, 125, and 127, became

infected. The other six were receiving *Micrococcus melitensis* in much smaller quantity and in association with other micro-organisms contained in urine, and of these only two, Nos. 122 and 123, became infected. Man, judging from the few cases of accidental experimental infection which have been published, is much more susceptible. Variation of susceptibility in different monkeys is also shown by the many groups in which, such as Section I. (c), identical procedure has resulted in the infection of some monkeys and not of others.

An interesting feature, which is brought out by a comparison of the incubation periods of the monkeys which had been infected by a pure emulsion of *Micrococcus melitensis*, is the difference in period according as the inoculation was a subcutaneous injection or absorption through an unbroken mucous membrane, it being decidedly shorter in the first than in the second group (reckoned to day of commencement of wave of fever), as shown in the following table :—

Subcutaneous inoculation.		Inoculation through unbroken mucous membrane.		
Serial number of monkey.	Incubation period.	Serial number of monkey.	Mucous membrane of—	Incubation period.
78	3	61A	Conjunctiva	15
79	5	100	Conjunctiva	14
80	4	48	Trachea	15
27	5	118	Nose	14
37	3			
38	2			

To sum up the foregoing experiments—

Dust Infected with Pure Culture of Micrococcus melitensis.—Nine monkeys experimented on. Five remained uninfected, four became infected—two through the eyes and two through the nose.

Dust infected with urine naturally containing Micrococcus melitensis.—Four monkeys experimented on; none became infected.

Contact Infection.—Two uninfected monkeys placed in *limited* contact with infected monkeys. Neither became infected. Infection by skin and skin parasites failed.

Two uninfected monkeys placed in *unlimited* contact with infected monkeys. One became infected, presumably through urine of infected animals, the other remained uninfected.

Infection through various unbroken mucous membranes with emulsion of Micrococcus melitensis.—All monkeys experimented on through conjunctival, tracheal, and nasal mucous membranes became infected.

Infection by Mosquitoes.—The monkey bitten every other day by

presumably infected mosquitoes for a period of two months did not become infected.

Infection by Food.—Three out of four monkeys given food contaminated by pure culture of *Micrococcus melitensis* became infected. Two out of six monkeys given food contaminated with urine from cases of Mediterranean Fever naturally containing *Micrococcus melitensis* became infected when dust was used as a vehicle.

Spleen pulp direct from a fatal case of Mediterranean Fever in man is not excessively virulent.

Subcutaneous infection by injection with hypodermic syringe of emulsion of pure culture of *Micrococcus melitensis* constantly produces infection with a very short incubation period. Urine from Mediterranean Fever cases naturally containing *Micrococcus melitensis* produces infection when injected subcutaneously into a monkey. Presumably, then, such urine could infect anyone engaged in nursing or who otherwise came in contact with it, through any existing scratch or abrasion of the cuticle.

Conclusions.

(1) The possibility of infection through the eyes and nose by means of highly infected dust has been experimentally demonstrated.

(2) The possibility of infection by unlimited contact has been experimentally demonstrated.

(3) The possibility of infection through food contaminated (*a*) with pure cultures of *Micrococcus melitensis*, (*b*) with dust soiled with urine naturally containing *Micrococcus melitensis*, has been experimentally demonstrated.

(4) The possibility of infection by urine of patients, through skin scratches, wounds, and abrasions, has been experimentally demonstrated.

II. IMMUNITY, SERUM, TOXIN, AND VACCINE EXPERIMENTS ON MONKEYS WITH REGARD TO MEDITERRANEAN FEVER.

By Staff-Surgeon E. A. Shaw, R.N.

Immunity.

The question as to whether an attack of Mediterranean Fever confers any protection against a subsequent exposure to infection is a very important one, and I determined to examine it—at any rate in monkeys—experimentally. As it was essential to have absolute proof of the first infection, a method had to be devised of securing this without sacrificing the monkey intended to be later subjected to a further infection. To recover *Micrococcus melitensis* from the blood of the experimental animal during life seemed the simplest way of doing this. The method described by Dr. Zammit* of doing this had not in my hands given satisfactory results. This I put down to two things, the small quantity of blood obtained by it and the risk of the blood taking up some disinfectant from the chemically-disinfected surface of the ear from which it was collected.

After carefully examining several monkeys, I considered it would be feasible to obtain blood to the amount of several cubic centimetres from a fairly large vein I found running superficially along the back of the calf muscles, more or less comparable to the external saphenous vein in the human subject. An attempt was first made to tap this with a small serum syringe, but this, the needle being too large relatively to the vein, was not successful. A sterile glass pipette was then taken with a constriction separating a containing bulb from the large open end, which was lightly plugged with cotton wool and fitted with a rubber tube, terminating at its free extremity in a glass tube mouthpiece. The capillary end of the pipette was drawn out fine in the peep light of a Bunsen burner, and the fine hair-like tube thus made broken through so as to give a fine terminal extremity of about a $\frac{1}{4}$ of an inch long. The skin just over the vein was drawn sideways and punctured with a sterile needle, and then allowed to resume its original position, so that the puncture came just over the vein; the mouthpiece of the rubber tube having been placed in one's mouth, the fine terminal end of the associated pipette was pushed obliquely into the vein through the small skin puncture previously made. When the point of the pipette was actually in the vein, blood immediately made its appearance in the capillary portion of the pipette, and it was found possible by gentle aspiration to get from 2 to 3 c.c. of blood into the containing chamber, whence it could be tranferred to broth tubes,

* Vol. 1, p. 89 of these Reports.

incubated and then subcultured on to agar slopes. In doing this on the infected monkey, the hair was cut close to the skin with scissors for about an inch square in the desired situation, then a pad dipped in a solution of lysol was applied to this area for 1½ hours, then removed, and the skin allowed to dry, a rough tourniquet lightly applied at the knee; and when the vein was distended it was tapped as described.

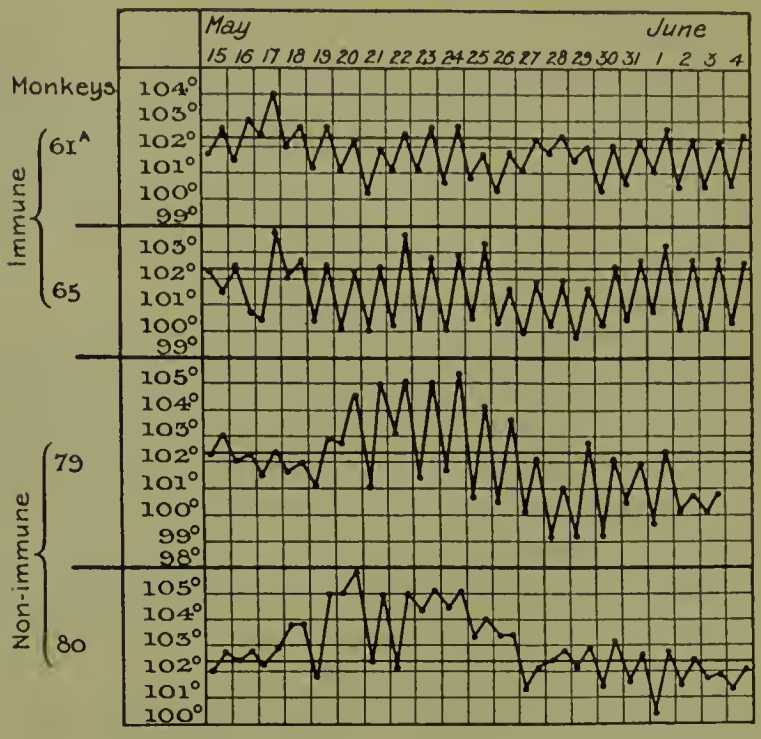
This procedure was first applied by me to an infected monkey (No. 72) on October 10, 1904, and *Micrococcus melitensis* was recovered from the blood thus obtained. I and other members of the Commission to whom I demonstrated it have since applied this method successfully to many other infected monkeys. The most favourable period at which to make use of it on an infected monkey would appear to be in the early stage of the fever, directly after the agglutination reaction has appeared, and when the top of the usual wave of fever has just been reached.

I expected in this way to have quite a number of monkeys on which to determine if one attack of fever conferred any protection against a second infection. But these monkeys, under the conditions of captivity which we were compelled to adopt, are not long lived, and most of them died at varying periods of from one to three months after the primary infection. This does not appear to have been necessarily secondary to their infection; the same mortality prevailed among the unused monkeys, from which subjects for experimentation were taken as required.

Experiment I.—In May, 1905, I had at my disposal Monkey No. 61A, which on December 19, 1904, had been infected with *Micrococcus melitensis* through the conjunctival mucous membrane, and from whose peripheral blood, taken on January 14, 1905, this micro-organism had been recovered; and also Monkey No. 65, which on January 25 had been dust-infected by the nose, and from whose blood, taken on February 14, *Micrococcus melitensis* had been recovered. Two unused monkeys, Nos. 79 and 80, had also been under preliminary observation in order to serve as controls.

May 15.—A three-day growth of a single stroke inoculation on agar of third generation of *Micrococcus melitensis* from human spleen was emulsified in 5 c.c. of sterile normal salt solution, and each of these four monkeys received 1 c.c. of this subcutaneously between the shoulders. I append a comparative chart showing for 21 days, starting from the date of injection (May 15), the curves of temperature of each of these four monkeys, with the usual base line ruled in at 102°·4.* There was no further rise in any of them after last date charted. The uninfected monkeys, Nos. 79 and 80, show a marked wave of fever commencing between the fourth and fifth day after the subcutaneous

* See charts in Part I of these Reports.



injection of *Micrococcus melitensis*. The presumably recovered monkeys, 61A and 65, show no such disturbance. Monkey No. 79 died on June 4, and *Micrococcus melitensis* was recovered from its organs *post-mortem*, thus demonstrating the activity of the strain of *Micrococcus melitensis* used.

The agglutinations were as follows :—

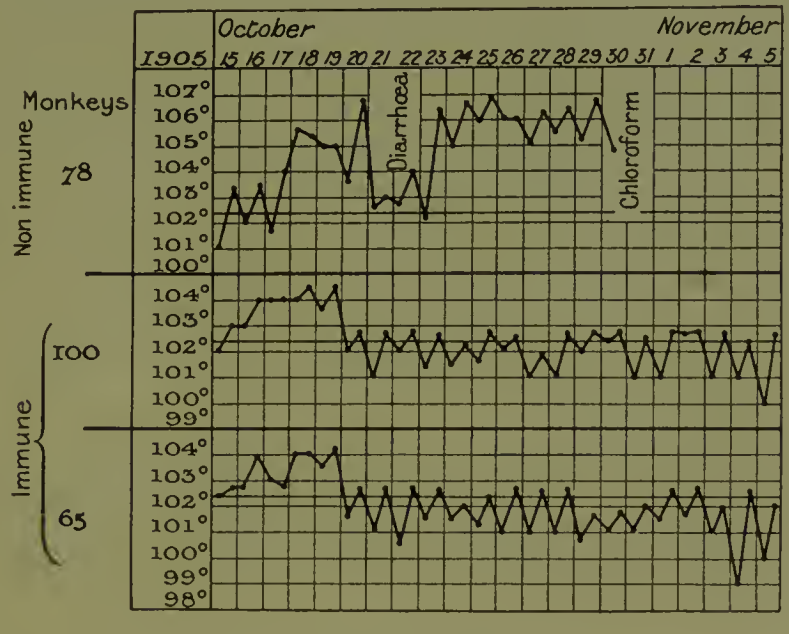
Date.	Monkey No. 61A.	Monkey No. 65.	Monkey No. 79.	Monkey No. 80.
April 25	1 in 500	1 in 250	Nil	Nil
May 15	1 in 500	1 in 200	Nil	Nil
May 22	1 in 1000	1 in 800	1 in 1000	1 in 1500
May 29	1 in 1000	1 in 600	1 in 800	1 in 3000
June 5	1 in 1000	1 in 800	Dead	1 in 2500
June 12	1 in 800	1 in 400	—	1 in 2500
June 18	1 in 1800	1 in 500	—	1 in 3000
June 25	1 in 800	1 in 400	—	1 in 2000
July 2	1 in 1000	1 in 1000	—	1 in 3000

Showing an increased agglutination in 61A and 65 following the subcutaneous injection, but a much greater response in this direction on the part of one of the previously uninfected monkeys (80). Blood was taken from three of these monkeys, 61A, 65, and 80 (79 was dead) on June 25 in the manner described, and incubated in broth; that from 61A and 65 did not yield *Micrococcus melitensis*, that from 80 did. This was repeated with Monkeys Nos. 65 and 80 on July 11 (61A was

suffering from bad diarrhœa), and again *Micrococcus melitensis* was recovered from 80 but not from 65.

Experiment II.—In October, 1905, I had available for repetition of the foregoing experiment Monkey No. 100, which had been infected during the preceding June through the conjunctiva and from whose peripheral blood *Micrococcus melitensis* had been recovered; and also Monkey No. 65, the same previously-infected monkey mentioned in the last experiment; Monkey No. 78, which had been under observation since the preceding May, and had never had the fever, was taken as a control.

October 15.—Each of these three monkeys received subcutaneously between the shoulders 1 c.c. of an emulsion of a five-day third generation growth of *Micrococcus melitensis* isolated from the human



spleen. The accompanying chart shows the temperature curves of all three starting from the date of the subcutaneous injection of *Micrococcus melitensis*. The very marked wave of fever in No. 78 contrasts conspicuously with the comparatively slight disturbance in Monkeys Nos. 100 and 65, the temperatures of which never rose after the last date charted.

The drop in the fever on October 21 and 22 in the case of Monkey No. 78 may possibly have been due to a sharp attack of diarrhœa which it developed at that time.

As I wished to cut and stain sections of infected organs, and as by October 30 there was every reason to think that the system of Monkey No. 78 was saturated with *Micrococcus melitensis*, it was on this date chloroformed, and the usual *post-mortem* inoculations were made; later, *Micrococcus melitensis* was recovered and verified in greatest profusion from its viscera, lymphatic, and salivary glands.

The agglutination reactions were as follows:—

Date.	Monkey No. 78.	Monkey No. 100.	Monkey No. 65.
October 15	Nil	1 in 800	1 in 40
October 18	Nil	1 in 800	1 in 40
October 21	1 in 30	—	—
October 22	1 in 320	1 in 800	1 in 40
October 25	1 in 1500	—	—
October 29	1 in 3000	1 in 1500	1 in 40
November 5	Dead	1 in 1500	1 in 40
November 12	—	1 in 1500	1 in 40

Showing much the same features as in the preceding experiment.

Though the resistance on the part of the immune Monkeys Nos. 61A, 65, and 100, does not appear to have been absolute, as in each there was a slight disturbance of temperature following the injection of *Micrococcus melitensis*, it must be remembered that in these experiments enormous numbers of *Micrococcus melitensis* were injected, an artificial infection being induced to a degree out of all proportion to one in the least likely to be met with in Nature. Also it has been shown in other microbic diseases in animals that immunity due to a previous attack can be overcome if a sufficient quantity of the responsible micro-organism be injected.

Conclusion.—Monkeys which had previously had an attack of Mediterranean Fever react much less markedly than others which have not to the same dose of living *Micrococcus melitensis*; *ergo*, a previous attack confers some protection against a subsequent exposure to infection.

A Therapeutic Serum.

Having two goats available which had been frequently subjected by me to subcutaneous injections of *Micrococcus melitensis* in large quantity, and whose blood gave a constantly high agglutination reaction, I determined to ascertain if the serum of one of these would have any effect on the course of the fever experimentally induced in a monkey. The goat selected to supply serum was the one with the higher agglutination, 1 in 3000; it has been already mentioned as the "white kid" in experiments described by me in Vol. 4 of these Reports, but at this period was mature.

On February 4, 1906, this goat's serum agglutinated 1 in 3000. Its last previous injection of *Micrococcus melitensis* had been given two months prior to this. On February 7 the goat's neck was shaved and sterilised over a selected area, and 5 c.c. of blood, taken from its right external jugular vein with a sterile serum syringe, were put in a flask with 80 c.c. of broth, and incubated. *Micrococcus melitensis* was

subsequently recovered and verified from this. Secondly, about half a litre of blood was run off from the same vein at the same time into a previously prepared sterilised conical filtration flask provided with a side tube, the nozzle of the serum syringe being replaced in the needle with a sterilised metal nozzle which had been fitted to a rubber tube connected with a glass tube which ran through an indiarubber cork in the neck of the flask to very near its bottom. The desired quantity of blood having been obtained, a spring clamp was applied to the rubber tube, the apparatus removed and placed in a dark cupboard. Next day the resulting serum was decanted through the side tube into a small conical sterilised flask. At the same time some of it was removed with a sterile pipette, and 1 c.c. was distributed over six agar plates, which were incubated at 37° C., and gave no growth of any kind after six days' incubation. This would indicate that *Micrococcus melitensis* in the circulating blood is contained in the corpuscles rather than in the fluid portion of the blood.

In readiness for the experiment two monkeys, Nos. 37 and 38, had been kept under preliminary observation for a week.

On *February* 3, 1906, each monkey received simultaneously between the shoulders and on the right side an injection of 1 c.c. of an emulsion of *Micrococcus melitensis* (first generation, fifth day growth from human spleen—Dakin), representing 1/6 of a single stroke inoculation of an agar slope.

February 9.—On this date the fever was well established in both monkeys, but rather more markedly in 37 than in 38; the former was therefore selected to receive hypodermically 3 c.c. of the serum obtained from the white goat as already described, the latter remaining untreated to afford a comparison.

February 10.—Monkey No. 37 received another hypodermic injection of the same goat's serum, this time of 5 c.c.

February 11	Agglutination was, in	Monkey 37, 1 in	160.
February 11	„	„ Monkey 38, 1 in	600.
February 18	„	„ Monkey 37, 1 in	500.
February 18	„	„ Monkey 38, 1 in	1500.
February 25	„	„ Monkey 37, 1 in	500.

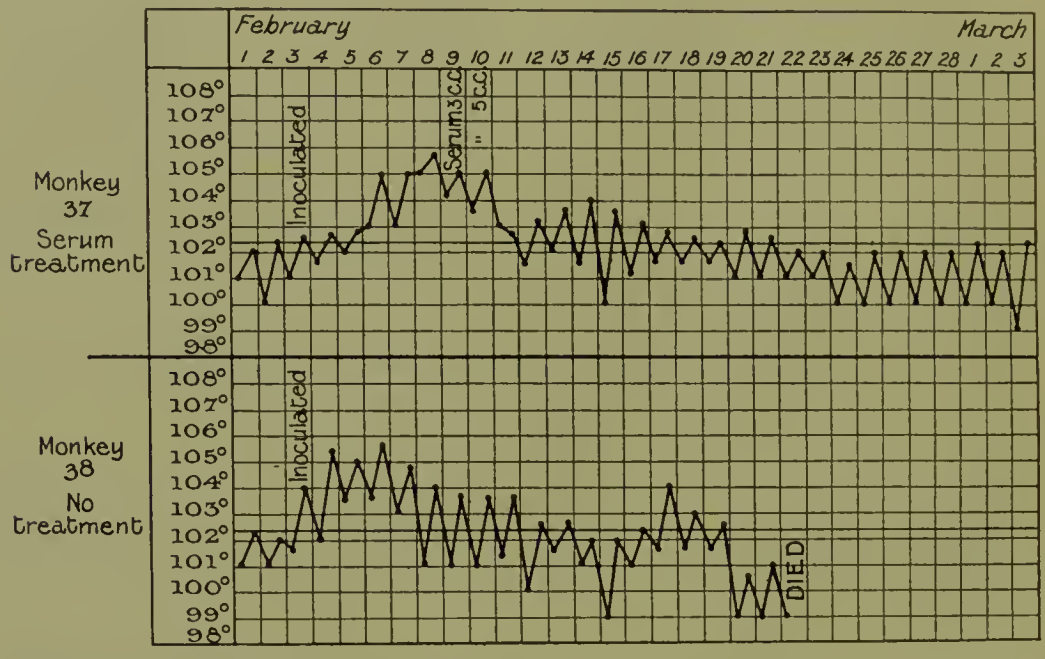
February 22 ..... Monkey 38 died.
March 9 ..... Monkey 37 died.

In both animals the usual *post-mortem* inoculations of slopes and plates were made, and as *Micrococcus melitensis* had not been previously recovered from the brain of monkeys, four plates were prepared from a similar amount of brain substance in each taken from the right parietal convolutions. In each case there was an abundant recovery of *Micrococcus melitensis* from the spleen and lymphatic glands, a less

plentiful recovery from the liver, and a still more restricted recovery from the kidneys; but there was no appreciable difference in number of colonies of *Micrococcus melitensis* in the two cases.

Micrococcus melitensis was recovered from the brain of both, 12 colonies in the case of Monkey No. 37 and 5 colonies in Monkey No. 38.

I append charts showing the course of temperature and dates of injections in each.



Result.—It will be seen that there is no such difference between the clinical features of these two cases of Mediterranean Fever in monkeys as to encourage a strong belief in the future successful development of a therapeutic serum.

Toxins.

To determine whether toxins of *Micrococcus melitensis* produce on monkeys the same clinical phenomena as living cultures.

Experiment I.—A flask containing 100 c.c. of nutrient broth was inoculated with a culture of *Micrococcus melitensis* derived from a Mediterranean Fever patient's blood; this was incubated for 21 days, then passed through a sterilised Chamberland F candle into a sterilised flask. Agar slopes were inoculated with the filtrate and incubated at 37° C. for four days; there was no growth of any description on these.

Two monkeys, Nos. 58 and 59, which had been under previous observations to determine freedom from infection, then received peritoneal injections with a serum syringe of the above filtrate as follows, No. 59, the somewhat larger animal, receiving a rather larger dose than the other:—

Date.	Monkey No. 58.	Monkey No. 59.
1904—		
August 8	5 c.c. of filtered toxins	7 c.c. of filtered toxins
August 9	Diarrhœa. Nil	7½ „ „ „
August 10	Diarrhœa better. Nil	6 „ „ „
August 11	5 c.c. of filtered toxins	6½ „ „ „
August 12	6 „ „ „	7 „ „ „
August 13	5 „ „ „	6 „ „ „
August 14	5 „ „ „	6 „ „ „

This procedure was followed by a slight rise of temperature, which began the day after the first injection in each case and lasted until the third day after the injections ceased in the case of No. 59, and until the fourth day in the case of No. 58, after which it dropped. This rise varied from day to day from 1° to 1°·5 F.

The agglutination reaction first appeared in No. 58 in a dilution of 1 in 20 on August 19, and was 1 in 20 August 24, 1 in 40 August 30, 1 in 80 September 5, remaining at this until the last time it was taken, September 29. The animal was chloroformed on October 2 and the usual *post-mortem* inoculations were made. No *Micrococcus melitensis* was recovered. In Monkey No. 59 the agglutination reaction first appeared August 24 in a dilution of 1 in 20, was 1 in 20 August 30, and 1 in 80 September 5. The animal was chloroformed on September 11 and the usual *post-mortem* inoculations were made. *Micrococcus melitensis* was not recovered.

Experiment II.—In this case it was decided to use broth cultures of *Micrococcus melitensis* killed by a heat of 60° C., instead of eliminating the micro-organism by filtration.

On July 22, 1905, a 60-c.c. broth flask was inoculated with *Micrococcus melitensis* recovered from an infected monkey; this was incubated at 37° C. till July 29, when a sub-culture (A) on to three agar slopes was made, the flask was then heated in a water-bath to 60° C., at which temperature it was kept for 30 minutes; then a sub-culture (B) was made also on three agar slopes. On August 1 the sub-culture A showed a plentiful growth of pure *Micrococcus melitensis;* the sub-cultures B were sterile.

Two monkeys, Nos. 116 and 117, which had been under preliminary observation, were subjected subcutaneously between the shoulders with this dead broth culture of *Micrococcus melitensis*, each receiving 5 c.c. on August 1 and 10 c.c. on August 19.

The agglutination reaction first appeared in Monkey No. 117 on August 6 in a dilution of 1 in 30, being 1 in 160 on August 9, 1 in 160 on August 13; highest, 1 in 400, on August 27; then falling away to 1 in 100 on September 10 and 1 in 40 on October 29. On August 13

blood was taken from the external saphenous vein and incubated in broth. No *Micrococcus melitensis* was recovered from this.

The agglutination reaction appeared in No. 116 on August 9 in a dilution of 1 in 30, which was never exceeded. The reaction was last found on September 6, and never appeared again, though examined for twice weekly till October 4, and thereafter once weekly till October 29. Blood was taken from the external saphenous vein and incubated in broth on August 17. *Micrococcus melitensis* was not recovered from this.

The course of the temperature in both these monkeys was precisely similar to that in the preceding experiment. There was a slight rise of temperature following each injection, but no wave of fever.

Experiment III (a).—Thinking that possibly there might be some difference between the results obtained by using dead broth cultures of *Micrococcus melitensis* as compared with those obtained by using dead agar slope cultures, I decided to repeat the experiment with the latter.

Accordingly, one agar slope of a six-day growth of *Micrococcus melitensis*, third generation, derived from human spleen, was emulsified in sterile normal salt solution and killed by heating in a water bath to 70° C. for an hour. I may mention here that this strain of *Micrococcus melitensis* so treated survived heating for half-an-hour to 65° C. This dead emulsion was taken up in a serum syringe and injected subcutaneously between the shoulders of two normal monkeys, Nos. 31 and 32, on November 4, 1905, each receiving half, that is the equivalent of half the product of one agar slope.

In both monkeys the agglutination reaction was first obtained on November 9.

Date.	Monkey No. 31.	Monkey No. 32.
November 9	1 in 30	1 in 30
November 12	1 in 150	1 in 100
November 19	1 in 250	1 in 400
November 26	1 in 250	1 in 400

In both there was a trifling elevation of temperature, but no wave of fever.

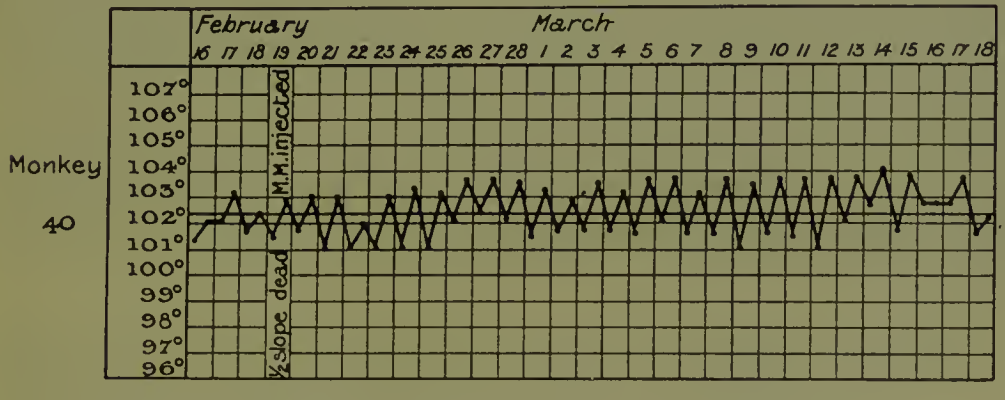
Experiment III. (b).—The foregoing procedure was repeated on two other normal monkeys, Nos. 39 and 40, using one agar slope of a four-day growth of *Micrococcus melitensis*, second generation, derived from human spleen, on February 19, 1906.

In both monkeys the agglutination reaction was first obtained on February 25, as follows :—

Date.	Monkey No. 39.	Monkey No. 40.
February 25.....	1 in 30	1 in 50
March 4	1 in 500	1 in 500
March 11	1 in 1000	1 in 1500

In both there was a trifling elevation of temperature, but no wave of fever.

Remarks.—In none of these eight monkeys was any fever produced as the result of these injections, though a slight rise of temperature was observable. I append a chart of Monkey No. 40, which is typical of the others.



In the case of the two monkeys injected with filtered broth cultures, low agglutination reactions were developed, 1 in 20 and 1 in 80 being the highest respectively developed.

In the case of the monkeys injected with broth cultures killed by heat, higher agglutinations were developed, 1 in 30 and 1 in 400 being the highest respectively obtained.

In the case of monkeys injected with heat-killed agar cultures, the highest agglutination reactions of all were realised, 1 in 250, 1 in 400, 1 in 1000, and 1 in 1500 being the highest respectively developed, closely approximating to those obtained in the case of monkeys injected with living *Micrococcus melitensis.*

Conclusion.—The toxins of *Micrococcus melitensis* do not produce the same clinical phenomena as living *Micrococcus melitensis*, no fever being developed, and the production of agglutinins being less.

A Possible Protective Vaccine.

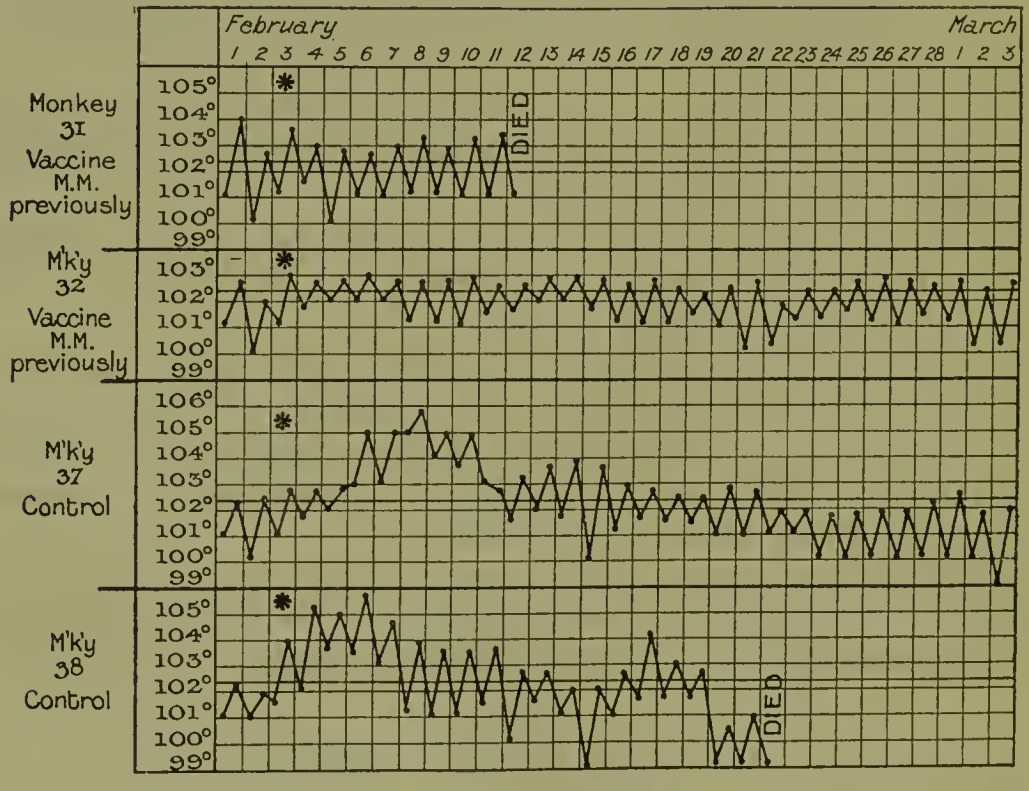
The immunity experiments having indicated that one attack of fever conferred a certain degree of protection against subsequent infection, it remained to be seen if the subcutaneous injection of dead cultures of *Micrococcus melitensis* developed any degree of protection.

As injections of dead agar cultures developed much higher reactions than did dead broth cultures, indicating a higher degree of reaction on the part of the organism to the former than to the latter, the monkeys injected with agar cultures killed by heat were taken for experimentation.

Monkeys Nos. 31 *and* 32, which had each on November 4, 1905, received half of the then heat-killed six-day *Micrococcus melitensis* growth of one agar slope as described in the preceding section, each on December 5 received the growth from one agar slope similarly prepared; and on December 30 this was identically repeated. These subsequent injections never caused any fever. The agglutination reactions follow later.

On the morning of February 3 each of these two monkeys received subcutaneously one-sixth of the growth of *Micrococcus melitensis* from one agar slope, first generation, from human spleen incubated for five days; two normal monkeys, Nos. 37 and 38, each receiving at the same time the same dose to act as controls.

I attach a comparative chart showing the subsequent course of temperature in all four, from which it will be seen that while each control monkey developed a typical wave of fever, neither of the vaccine monkeys did so.



* On this date, February 3, each monkey received the same dose of living *Micrococcus melitensis*.

The course of agglutination in the four monkeys was as follows:—

Date.	Monkey No. 31.	Monkey No. 32.	Monkey No. 37.	Monkey No. 38.
November 19	1 in 250	1 in 400	No observation	No observation
November 26	1 in 250	1 in 400		
December 3	1 in 100	1 in 200		
December 5	2nd injection of dead *Micrococcus melitensis*.			
December 10	1 in 50	1 in 200		
December 24	1 in 40	1 in 100		
December 30	3rd injection of dead *Micrococcus melitensis*.			
December 31	1 in 40	1 in 240		
January 7.....	1 in 240	1 in 400		
January 14.....	1 in 300	1 in 800		
January 21.....	1 in 300	1 in 400	Nil	Nil
January 28.....	1 in 500	1 in 800	Nil	Nil
February 3	1st injection of living *Micrococcus melitensis*.			
February 4	1 in 600	1 in 700	Nil	Nil
February 7	1 in 1200	1 in 800	Nil	1 in 30
February 11	1 in 600	1 in 1200	1 in 160	1 in 600
February 12	Died	—		
February 18	—	1 in 1000	1 in 500	1 in 1500
February 22	—	—		Died
February 25	—	1 in 1200	1 in 800	
March 9..... ...	—	—	Died	
March 11.....	—	1 in 1000		

Monkey No. 31 on January 28, prior to its first injection with living *Micrococcus melitensis*, was noticed to be unwell.

February 4. The note is "still seedy, no diarrhœa."

February 11. Very "seedy," some diarrhœa; brought from terrace into laboratory for warmth.

February 12. Died. *Post-mortem*. Spleen enlarged. Miliary tuberculosis of lungs. Inoculations from all organs and lymph glands.

February 17. *Micrococcus melitensis* recovered from all glands and organs save kidneys.

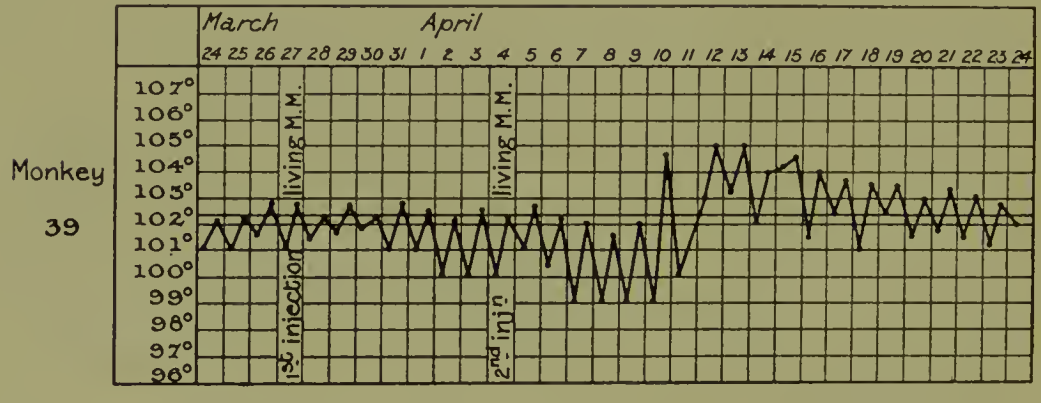
Monkeys Nos. 37 and 38, the controls. The history of these two animals will be found under the section on "A Therapeutic Serum."

Second Experiment.—Monkeys Nos. 39 and 40 were similarly treated with *Micrococcus melitensis* killed by heat, each receiving on February 19 half the product of one agar slope, a four-day growth, second generation, from human spleen (Dakin). This was repeated in each case on March 13.

Monkey No. 40.—Agglutination first appeared on February 25, six days after first injection of dead *Micrococcus melitensis*, in a limit dilution of 1 in 50, rising to 1 in 500 on March 4 and 1 in 1500 on March 11. It unfortunately died from dysentery on March 20. The usual *post-mortem* inoculations were made from all glands and

organs, but no *Micrococcus melitensis* was recovered, thus further verifying the previous death of the *Micrococcus melitensis* injected in both cases.

Monkey No. 39.—Agglutination reaction first appeared on February 25 in a limit dilution of 1 in 30, rising to 1 in 500 on March 4 and 1 in 1000 on March 11, falling after the second injection to 1 in 400 on March 25. On March 27 this monkey received 1 c.c. (representing one-fifth of one slope) of an emulsion of living *Micrococcus melitensis*, four-day growth, second generation, from human spleen (Dakin). On March 30 it appeared unwell and was brought from the terrace to live in a big box in the laboratory. Its agglutination reaction was 1 in 600 on April 1. Its temperature having remained undisturbed for eight days after the first injection of living *Micrococcus melitensis*, that is the outside incubation period for subcutaneous inoculation having been passed, it was decided to give it a second subcutaneous injection of living *Micrococcus melitensis* in order to see if the apparent resistance would be overcome or not. Accordingly on April 4 it received another dose precisely similar to that of March 27, and on April 10, exactly six days after, it commenced a wave of fever. Its resistance had been overcome by the additional dose of living *Micrococcus melitensis*.



The appended chart shows the temperature from a few days prior to the first injection of living *Micrococcus melitensis* and to the end of the wave of fever. It was recorded from February 16 onwards twice daily, remaining normal till April 10. The agglutination reaction was 1 in 500 on April 8 and 1 in 500 on April 15.

Remarks.—Considering the enormously large doses of living *Micrococcus melitensis* administered in these experiments without producing the characteristic wave of fever in the monkeys protected by previous injections of the dead organism, it must be conceded that there is an extremely hopeful outlook for the future of similar protective inoculations of mankind against this fever.

III. FURTHER OBSERVATIONS ON GOATS, CATS, RATS, AND AMBULATORY CASES IN CONNECTION WITH MEDITERRANEAN FEVER.

By Staff-Surgeon E. A. Shaw, R.N.

Goats.

The Advisory Committee having suggested the advisability of making observations to determine whether or not there existed a seasonal prevalence of Mediterranean Fever amongst goats such as exists amongst men, I proceeded during the first week in March, 1906, to re-examine the goats supplying milk to Bighi Hospital in order to be able to compare the winter prevalence then found with the summer prevalence previously ascertained by me in the same group of goats in June to July, 1905.

This examination was commenced on March 3 and finished on March 11. At this period 74 goats were supplying milk to this hospital as against 91 goats at the previous examination. The component herds were the same on both occasions, but the smaller number in the winter was due to the larger average yield of milk per goat at this period associated with a somewhat smaller number of patients in hospital. The result of this second examination of goats was as follows:—

Date of examination.	Serial number of goat.	Number of *Micrococcus melitensis* colonies recovered.
March 3, 1906	3	Innumerable
March 3, 1906	4	6 colonies
March 4, 1906	22	27 ,,
March 4, 1906	33	12 ,,
March 4, 1906	36	2 ,,
March 4, 1906	38	76 ,,
March 5, 1906	42	3 ,,
March 5, 1906	63	241 ,,

Thus in March eight goats out of 74, or 10·8 per centum, were found to contain living *Micrococcus melitensis* in their milk, as against nine out of 91, or 9·9 per centum, in June to July. There is thus no appreciable indication of a seasonal prevalence affecting this group of goats, but the number of animals here examined is small, and will have to be taken in connection with results obtained by the other workers who have been making a similar re-examination of the groups of goats examined by them last summer.

The history of the two experimental goats continued from my last

report* is briefly as follows:—The elder brown goat ♀ again received subcutaneously, on November 21, 1905, the emulsified six-day growth from six agar slopes of *Micrococcus melitensis* (human spleen growth, third generation); its agglutination was on this date 1 in 2500, on December 3, 1 in 4000, and on December 24, 1 in 3000. On December 26 it similarly received the five-day growth from two slopes of second generation from a race of *Micrococcus melitensis* very virulent for guinea-pigs, obtained from Dr. J. W. H. Eyre, of Guy's. This developed a "negative phase," the agglutination reaction dropping to 1 in 800 on December 31, rising to 1 in 1000 on January 7, 1906, and 1 in 2000 on January 14, 1 in 2000 on February 18, 1 in 3000 on March 11, and 1 in 3000 on April 15. This animal, not having been impregnated since its purchase in June, 1904, has yielded no milk since June, 1905, and has been treated as above described for the sake of its serum.

[*Note.*—On May 26, 1906, this goat, which was suffering from a localised subcutaneous abscess at the site of the last inoculation, was slaughtered.

Post-mortem.—Cultivations from spleen and mesenteric glands gave profuse growth of *Micrococcus melitensis*. Cultures from inguinal glands and the cheesy material from the abscess cavity remained sterile. Blood gave a serum reaction of 1 in 1000.]

An Impregnation and Natural Feeding Experiment.

The younger white goat ♀, now mature, was sent in October, 1905, to be impregnated. On November 21 it received the emulsified growth from four slopes of the same *Micrococcus melitensis* as its companion (*vide* supra); its agglutination was then 1 in 1000, 1 in 1000 on November 26, 1 in 800 on December 3, 1 in 1000 on December 10, 1 in 1500 on December 24. On December 26 it received one slope of same strain of *Micrococcus melitensis* as its companion. Its agglutination was 1 in 2000 on December 31, 1 in 1500 on January 7, 1906, 1 in 3000 on January 14, and 1 in 1500 on February 18. On February 7, 5 c.c. of blood were taken aseptically from its right external jugular vein and incubated in broth. *Micrococcus melitensis* was recovered and verified from this. On March 2 it was delivered of a kid; its agglutination on this date was 1 in 1000. The new-born kid's blood was taken and examined for agglutination reaction; this was found to be present in a limit dilution of 1 in 800, thus being slightly less than that of the mother. Agglutinins had thus passed from the maternal to the fœtal blood, *via* the placental circulation. It now remained to see if the *Micrococcus melitensis* demonstrated to have been present in the mother's blood during pregnancy had similarly entered the fœtal circulation. The kid, born March 2, was chloroformed on

* See Part IV of Commission Reports.

March 4, and the usual *post-mortem* inoculations made from the axillary femoral and mesenteric lymphatic glands, and from the spleen, liver, kidneys, urine, and heart's blood, 36 Petri dishes and 12 broth tubes being used for this purpose. *Micrococcus melitensis* was not recovered, the tubes and plates all being sterile with the exception of two which contained a few accidental, apparently air, contaminations.

Micrococcus melitensis did not appear to have passed into the fœtal circulation though present in the maternal blood.

[*Note.*—Up to June 27 the milk from the white goat was examined regularly twice a week, but *Micrococcus melitensis* was never detected in the plates prepared from it. The kid, whose blood had likewise been examined twice a week and invariably given a negative reaction when tested in dilutions of 1 in 10, 1 in 20, and 1 in 50, was removed from the white goat on June 17, 1906, and utilised for another experiment.]

On March 6 a new-born black kid from an uninfected mother was examined for agglutination reaction; this not being found present, it was placed with the bereaved mother goat to function as a natural infected milk-feeding experiment, and the two animals were isolated. The milk of the mother was plated twice weekly (four plates each time), commencing March 2. *Micrococcus melitensis* had not appeared in it by April 20, nor had the little kid ever presented any agglutination reaction, though this was periodically looked for. The mother's agglutination reaction went up to 1 in 2000 by March 11, and was 1 in 2000 on April 15.

In consequence of my impending departure from Malta, Dr. Eyre took over all three of these animals from me on April 21, and will continue the experiments.

Cats.

On February 24, 1906, I commenced an examination of cats to ascertain if Mediterranean Fever was to be found affecting them at all. There being no "home" for lost or strayed cats in Malta such as there is for dogs, considerable difficulty was encountered in getting animals in quantity for examination; they could only be got singly and at intervals of days. Up to April 21 I had succeeded in getting 22. Of these five presented an agglutination reaction to *Micrococcus melitensis* in a dilution of 1 in 30. I was able to purchase three of these, chloroformed them, and made the usual *post-mortem* inoculations, using in each case—

4 plates for femoral lymphatic glands.
4 „ axillary „ „
4 „ mesenteric „ „
4 „ spleen
4 „ kidneys
2 „ liver
6 broth tubes for heart's blood.

Micrococcus melitensis was recovered and verified from one cat only, and that in very small quantity, three colonies in one of the plates prepared from the mesenteric lymphatic glands. This cat belonged to the mother of one of the laboratory labourers, who was living in Birchircara, a small town three miles from Valletta, but in a house quite apart from that in which her son lived with his family.

Rats.

Early in March, 1906, I made arrangements for obtaining rats from the naval dockyard, the abattoir and the main drains in order to ascertain if these were at all infected with Mediterranean Fever. This examination was commenced on March 7, and up to April 21 I had examined the blood of 43 rats, all of the common dark brownish-grey type usually found in sewers. Of these three presented a faint agglutination reaction in a dilution of 1 in 30 with *Micrococcus melitensis*, and were duly chloroformed and the usual *post-mortem* inoculations from glands and organs made, but no *Micrococcus melitensis* was recovered from any one of them. On March 22 I noticed trypanosomes in the blood of one rat, and have found them in the blood of eight rats out of 26 examined since that date. In one of these eight cases the parasites after staining appeared to be the typical ordinary *Tr. Lewisii* usually found in rats; in the other seven the parasites were all of similar length and breadth, relatively more slender than *Tr. Lewisii* and presenting no such variations of form as the latter usually does.

Ambulatory Cases.

I have continued the examination of two of the ambulatory cases* since their discovery in June, 1905, up till April 20, 1906, when I turned them over to Dr. Eyre on my impending departure from Malta.

These two cases were those (No. 9, B. Worley, and No. 11, F. Mallia) whose urine contained *Micrococcus melitensis* in such extraordinarily large quantities. The urines from both these cases have been collected and samples plated twice weekly throughout the period named, and they have never failed to contain *Micrococcus melitensis*. The amount, as shown in the previous Report, has been very variable. The average number of colonies found at each examination during each month for Case 9, B. Worley, has been as follows:—

1905—October	3000	colonies of *Micrococcus melitensis*	per	c.c.
November ...	2400	,,	,,	,,
December.....	4200	,,	,,	,,
1906—January	2800	,,	,,	,,
February	5400	,,	,,	,,
March	1200	,,	,,	,,
April.....	540	,,	,,	,,

* See Part IV of Commission Reports.

These numbers have been determined in the manner detailed in the previous Report. The other case, No. 11, F. Malhia, has been under treatment with drugs with a view to seeing if the excretion of living *Micrococcus melitensis* in his urine could be affected, Case 9 being left untreated to function in some degree as a standard of comparison. Urotropine was first tried, then hydrarg. perchlor.; each was given three times a day, the following table shows with what result:—

Dates of drug administration and dose.		Date of plating urine.	Number of colonies of *Micrococcus melitensis* recovered per cubic centimetre of urine.
		1905.	
Dec. 26 to Jan. 14...	2 grains of urotropine three times a day	December 26.....	1,200
		December 29.....	1,300
		1906.	
Jan. 15 to Jan. 22...	Increased to 15 grains t. d. s.	January 2	900
		January 5	3,600
		January 9	3,600
		January 12	3,600
		January 16	4,800
		January 19	4,800
Jan. 23 to Feb. 5 ...	Increased to 20 grains t. d. s.	January 23	2,400
		January 26	4,200
		January 30	3,000
		February 2	6,000
		February 6	6,000
		February 9	8,400
Feb. 6 to Feb. 26 ...	$\frac{1}{12}$th of a grain of hyd. perchlor. three times a day	February 13	3,600
		February 16	4,000
		February 20	2,400
		February 23	1,200
		February 26	2,000
		March 2	480
		March 6	5,400
Feb. 27 to Mar. 31...	Increased to $\frac{1}{6}$th of a grain t. d. s.	March 9	3,600
		March 13	12,000
		March 16	7,000
		March 20	5,000
		March 23	3,000
		March 26	4,800
		March 30	3,600
April	Treatment suspended	April 2.....	4,800
		April 6.....	1,200
		April 10.....	1,200
		April 14..... ...	Man on leave
		April 17.....	600

The number of colonies are approximate, being determined by counting colonies in an average area of 1 cm. square and multiplying out, $\frac{1}{6}$ of a c.c. being distributed over each plate.

It will be seen that the drugs used had practically no effect on the exeretion of the living *Micrococcus melitensis*, nor presumably, therefore, on its life in the human body. These two cases have been turned over to Dr. Eyre for further treatment and observations.

IV. *MICROCOCCUS MELITENSIS* AND ANTISERUM.

By J. W. H. Eyre, M.D.

(Received July 2, 1906.)

The first serious attempt to produce an antiserum for therapeutie use in Malta Fever was made by Wright, who, in 1895, treated goats and in the following year a horse by the subcutaneous injection of "killed" cultures of *Micrococcus melitensis*. The serum obtained from the goats appeared to possess but little agglutinative power, and when employed in the treatment of monkeys, either previously or subsequently to the injection of living cultures of the Micrococeus, exhibited neither proteetive nor curative properties. The serum of the horse was further used in the treatment of patients suffering from Malta Fever, and some of these human cases recorded by Aldridge* in 1898 showed, subsequently to the administration of the serum, improvement which was ascribed to the action of the serum. No further observations or experiments in this direction have, however, been recorded sinee. In 1903 I commenced a series of experiments dealing with the immunisation of rabbits and of guinea-pigs, in the hope of obtaining a bactericidal serum of demonstrable potency; next were tried goats, and finally, in 1905, I undertook the treatment of a horse. The results obtained to date are by no means so encouraging as was antieipated, but certain points have been estab-lished which help to elucidate phenomena observed during the course of experimental work on the Micrococcus, to which points it appears advisable to draw attention.

My early experiments, in which the rabbit as well as the guinea-pig was employed, confirmed Durham's† valuable observations; his results may be summarised as follows, the illustrative cases being taken from my own note-book:—

(*a*) That the development of specific agglutinins in the blood was

* 'Lancet,' vol. 1, 1898, p. 1394.

† 'Journ. of Path. and Bact.,' vol. 5, 1899, p. 377.

slower in rate and less in amount in the most severe and in the least severe infections (compare Animals Nos. 1 and 2, Table I). Speaking generally, the formation of large quantities of agglutinins took place when the resisting power of the infected animals was considerably but not *over* strained.

(*b*) That there was apparently little direct relationship between agglutinins and antitoxic or antibacterial substances, as the blood of infected animals frequently showed a high agglutinative power for some time prior to a fatal termination (see Animals Nos. 3, 4, and 5, Table I).

(*c*) And, finally, that animals whose blood at death possessed a low agglutination index often showed a general blood infection with abundant cocci, whilst it was frequently observed in those with high agglutinative power that cocci were either absent from the blood of the general circulation or present in very small numbers (compare Animals Nos. 1 and 3, Table I).

Occasionally, however, the cocci are present in the peripheral blood in enormous numbers, even when the sedimentation value of the serum is fairly high (see Animals Nos. 4 and 5, Table I).

Table I.

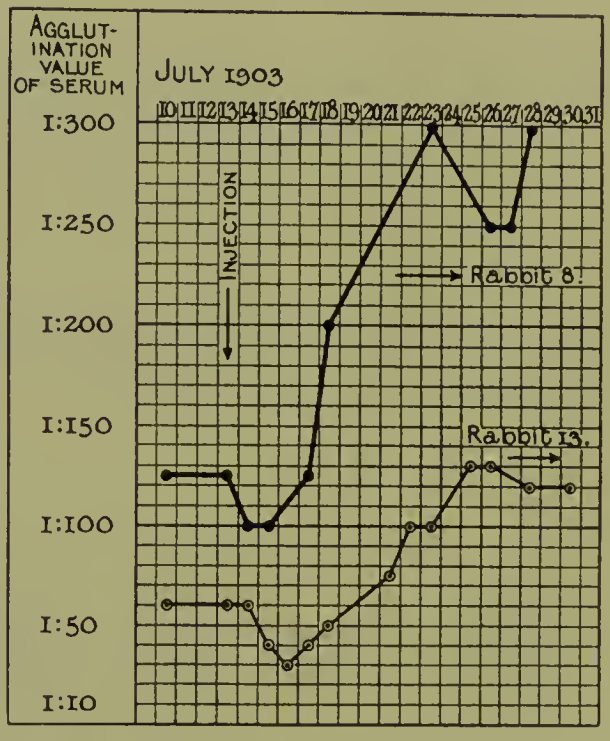
Reference No.	Guinea-pig No.	Dose of living culture.	Method of inoculation.	Sedimentation value of serum.	Interval between inoculation and death.	Number of cocci per 10 cm. heart blood at death.
		loop.				
1	63	1	Intracerebral	1 : 10—	10 hours	6000
2	12B	0·001	,,	1 : 500+	28 days	Nil
3	15	1	,,	1 : 1100+	27 hours	3
4	90	1	,,	1 : 600+	4 days	500
5	19C	1	,,	1 : 1000+	21 ,,	1000

In addition it was found that the intravenous injection of graduated doses of killed cultivations of *Micrococcus melitensis* provoked the formation of agglutinins (though, speaking in general terms, in lesser quantities than followed the injection of suitable doses of living cultures), and that after agglutinins had been formed in demonstrable quantities, the immediate effect of the introduction of a further dose of killed culture was to temporarily diminish the quantity of agglutinins present in the serum, and its more remote effect to provoke a marked increase (see Chart I). Again, it was often observed that if the injections were too frequently repeated, this immediate diminution was cumulative (see Chart II), conclusively showing that the

formation of agglutinating substances for *Micrococcus melitensis* followed the same laws as those of typhoid, dysentery, and other better-known agglutinins.

After the long-continued treatment of the rabbit by repeatedly injecting suitable doses of killed cultures, and the establishment and maintenance of a high agglutinative power in the blood, the introduction of even comparatively small amounts of living virulent cultures

Chart I.



Showing the immediate and remote effects upon the sedimentation index of the experimental rabbit of 2 milligrammes "killed" cultivation of *Micrococcus melitensis* administered intravenously.

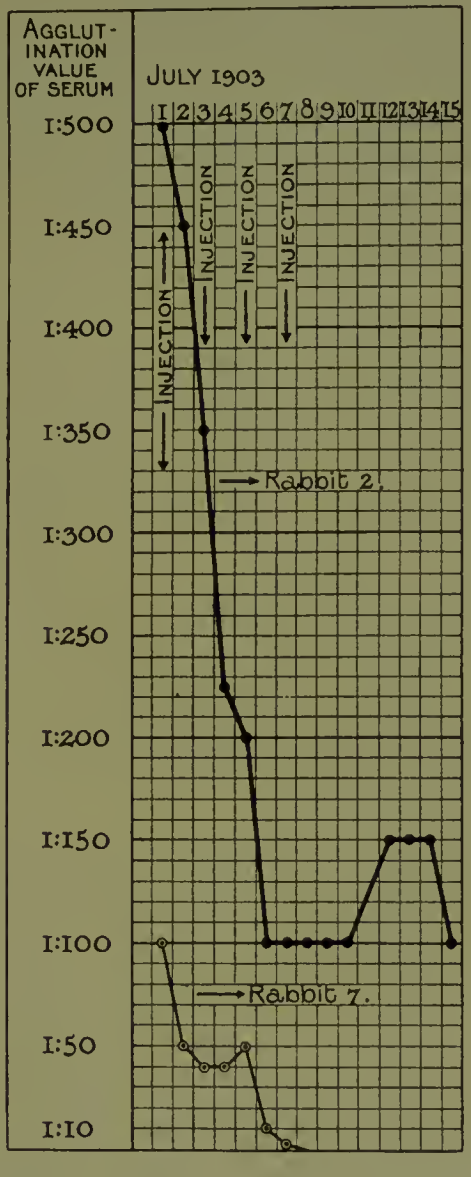
almost invariably caused the death of the animal, whilst the highly agglutinating blood serum of the treated animals failed to protect normal rabbits against infection or modify the course of such infection.

E.g., Rabbit No. 8, weight 3000 grammes, under treatment for seven months, litre of serum = 1 : 1500, received one loop of a three-day old agar culture of *Micrococcus melitensis* intracerebrally. Death occurred in 10 days from *Micrococcus melitensis* septicæmia, and *post-mortem* all the organs and tissues were found to be crowded with the micrococci.

The difficulties in the way of obtaining serum in sufficient quantity from the rabbit for extensive tests of its influence on the course of infections resulting from the injections of other animals with *Micrococcus melitensis* led me to attempt the immunisation of goats, employing at first the method of intravenous injection of killed cultures of *Micrococcus melitensis*. After five months' work it was

found that the agglutinative power of the blood serum could not be pushed much beyond 1 : 200, although the amount of inoculum introduced at the latter injections was equivalent to the entire bacterial growth of one Roux culture bottle. The subscquent injection

Chart II.



Showing the effects produced upon the sedimentation index of the experimental rabbit by the repeated injection of small doses of living cultivations of *Micrococcus melitensis* (0·001 of a loop).

Note.—Rabbit No. 2 died July 30. *Micrococcus melitensis* recovered in large numbers from all organs.

Rabbit No. 7 died August 28. *Micrococcus melitensis* not recovered.

intravenously of living cultures elicited no adequate response, although it yielded the further information that the coccus remained alive in the general circulation and could be recovered from the peripheral blood of the animal at least a month after injection. On the other hand, owing to the low virulence of the culture of *Micrococcus melitensis*

with which I was working, the course of the infection in the experimental animals extended to months, and it became impossible to ascribe therapeutic value to the goat's serum when an animal treated therewith survived the control by two or even three months—even when at the *post-mortem* examination cocci were absent from the organs, bone marrow, and urine, for similar findings were not infrequently recorded in the control guinea-pigs.

Under these circumstances I directed my attention to the exaltation of the virulence of the *Micrococcus melitensis* for the guinea-pig, with the result (as detailed in a previous paper in these Reports—Part II, p. 67) that a loop holding some 0·5 milligramme of culture could be depended to produce death within seven days. Such a dose, although obviously not the minimal fatal dose, is referred to as the "standard" dose.

A chestnut mare was purchased by the Commission at the end of March, 1905, and after satisfactorily passing the tuberculin and mallein tests its serum was tested against an emulsion of *Micrococcus melitensis* and was found to be totally devoid of agglutinative power on the coccus, even when equal quantities of the serum and emulsion (1 : 2) were placed in contact. Treatment was begun on April 3 by the subcutaneous injection of 10 milligrammes of *Micrococcus melitensis* culture, previously suspended in 10 c.c. saline solution and killed by heating to 59° C. for 30 minutes in water bath. In this connection I may mention that the strain of *Micrococcus melitensis* used throughout these horse injections was the one, highly virulent for guinea-pigs, mentioned above.

At intervals of about one week, the exact time being determined by attention to such points as the general condition of the mare, temperature, etc., the injection was repeated and the size of the dose of killed culture gradually increased until after nearly two months' treatment it had reached 250 milligrammes. The quantity of agglutinins formed in response to these injections was, however, small, and a complete reaction could not be obtained in higher dilutions than 1 : 5. The seat of inoculation was then changed, and 18 milligrammes of dead cocci injected directly into the external jugular vein, with the result that the agglutinins immediately increased in amount and the sedimentation curve rose to 1 : 100. Living cocci from three-day old agar cultivations were then substituted for the "killed" cultures, and were injected intravenously (gradually increasing doses from 5 milligrammes up to about 3000 milligrammes being introduced on 10 separate occasions during the following six and a-half months), and in this series the interval between the injections was regulated by the movements of the sedimentation curve, which invariably responded to an injection in the manner already referred to and graphically represented in Chart I. The

details of the 18 injections are summarised in the accompanying table (II).

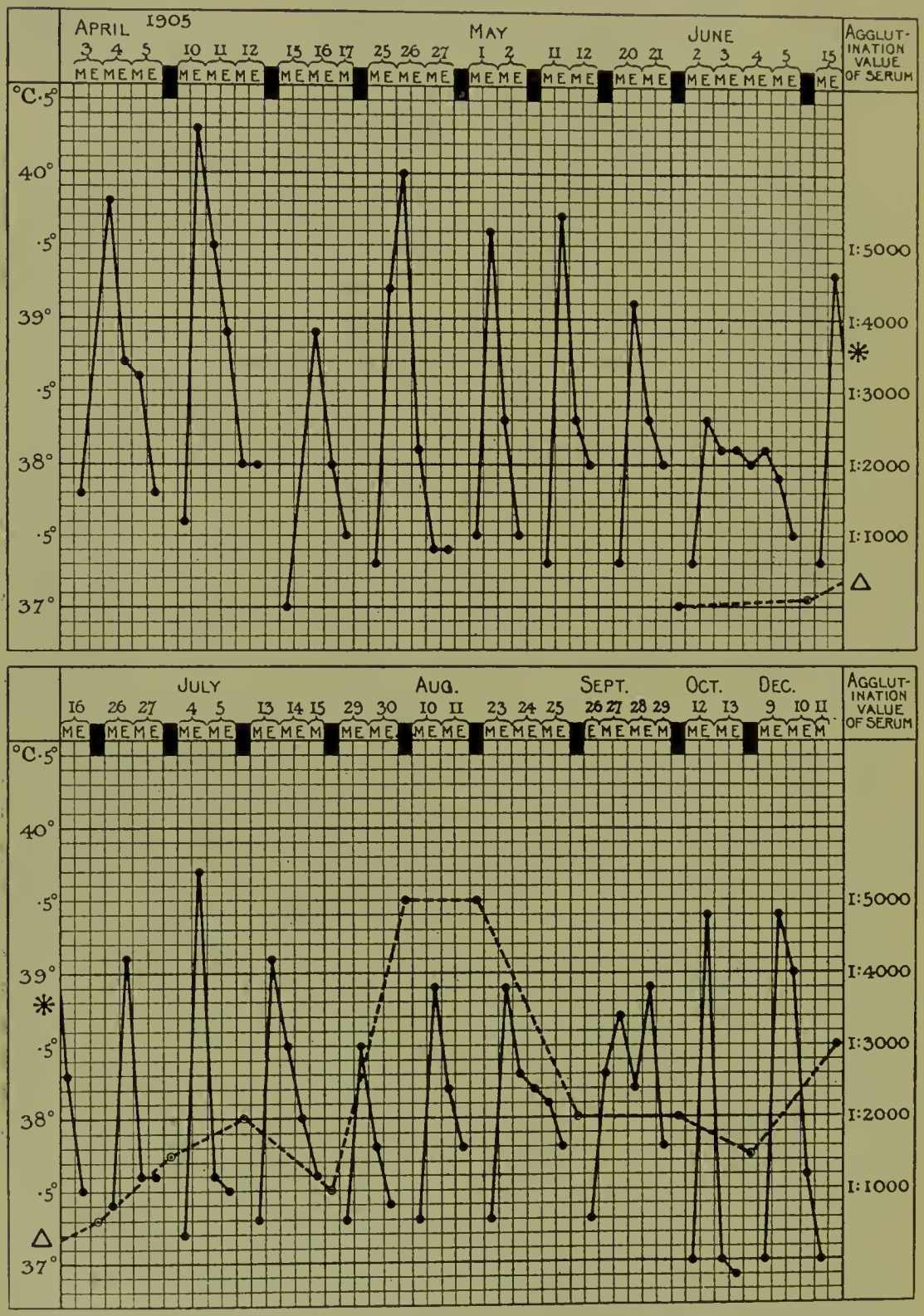
Table II.—Details of Horse Inoculations.

No. of inoculation.	Date.	Character of inoculum.	Approximate size of dose of cocci.	Bulk of emulsion injected.	Site of inoculation.	Resulting sedimentation value of serum.
			mg.	c.c.		
1	3/4/05	"Killed" cultivation	10	10	Subcutaneously	1 : 2 −
2	10/4/05	,,	25	10	,,	1 : 2 −
3	15/4/05	,,	25	10	,,	1 : 2 −
4	25/4/05	,,	50	20	,,	1 : 2 −
5	1/5/05	,,	100	50	,,	1 : 2 −
6	11/5/05	,,	100	50	,,	1 : 2 −
7	20/5/05	,,	250	50	,,	1 : 5 +
8	2/6/05	,,	18	10	Intravenously	1 : 100 +
9	15/6/05	Living cultures	5	20	Intravenously	1 : 600 +
10	26/6/05	,,	5	10	,,	1 : 1500 +
11	4/7/05	,,	10	25	,,	1 : 2000 +
12	13/7/05	,,	25	50	,,	1 : 1000 +
13	29/7/05	,,	25	50	,,	1 : 5000 +
14	10/8/05	,,	50	50	,,	1 : 5000 +
15	23/8/05	,,	50	50	,,	1 : 2000 +
16	26/9/05	,,	1250	50	,,	1 : 2000 ±
17	12/10/05	,,	1250	50	,,	1 : 1500 +
18	9/12/05	,,	3000	100	,,	1 : 3000 +

The clinical phenomena exhibited by the animal subsequently to an injection were remarkably few. The temperature invariably rose within a few hours of the injection, but rarely more than 1°·5 to 2° C., and the mare was "off her feed" for perhaps 24 to 36 hours. The temperature rapidly fell, and was again normal within two or three days. After a subcutaneous inoculation of the dead bodies of the cocci a small local swelling appeared in about 12 to 18 hours, which was tender and "boggy" to the touch. This was soon absorbed as a rule, but on one occasion it persisted for some days, became conical in shape, and the apex of the cone became so soft as to induce me to incise at this point. No pus, however, was present; cultures from the œdematous subcutaneous tissue remained sterile, and the wound rapidly healed. On another occasion the emulsion of living cocci was prepared with sterile distilled water instead of normal saline solution and injected intravenously, with the result that a certain amount of thickening occurred along the course of the external jugular vein, requiring nearly a week for its complete absorption, and causing a heavy fall in the sedimentation value of the serum.

Beyond these two mishaps nothing occurred to disturb the progress of treatment, and in February, 1906, some six weeks after the final bleeding, the mare had immensely improved in weight, general appearance, and spirit since her purchase by the Commission.

Chart III.



Showing the response of the temperature (continuous line) and sedimentation (interrupted line) curves of the Malta Fever mare to each of the 18 injections. The average temperature of the normal horse is 37°·57 C. (Sims Woodhead, 'Proc. Physiological Soc.,' vol. 23, 1899, pp. 15—18.

In the accompanying chart I have abstracted the movements of the curves of the temperature, and of the sedimentation value of the serum, corresponding to each individual inoculation.

In addition to the samples of blood frequently abstracted to determine the amount of agglutinins present, larger quantities (some 250 c.c.) were drawn at intervals, and on the separation of the serum tests were made in the first place of its sterility and in the second of its protective properties. With reference to the first point, so far as could be determined by plate and tube cultivations, the serum, when carefully decanted from the blood clot, was absolutely sterile, but the injection of animals showed that a sufficient number of micrococci were present in the blood serum when this had been drawn within about three weeks of an inoculation to cause a fairly acute infection, but that by the end of about four weeks after injection, the horse had been able to remove all living cocci from the general circulation, and the serum was then innocuous and presumably sterile.

These results may be conveniently tabulated as follows :—

Table III.

Date.	Animal and number.	Injected with horse serum.		Method of inoculation.	Result.
		Quantity.	Obtained—days since last injection.		
		c.c.			
23/8/05	Guinea pig 16	10	13	Subcutaneously	Death in 3 days, *M. melitensis* recovered.
,,	,, 57	10	13	,,	Death in 2 days, *M. melitensis* recovered.
15/9/05	,, 64	10	21	,,	Death in 3 days, *M. melitensis* recovered.
22/9/05	,, 16A	10	28	,,	Animal unaffected—serum absorbed.
24/11/05	,, 17	10	28	,,	Animal unaffected—serum absorbed.
,,	Rabbit 171 ...	10	28	Intravenously	Animal unaffected.

Note.—It was probably during the dilution of the serum drawn on August 20 in readiness for me to determine its sedimentation value, or during the performance of the *post-mortem* on guinea-pigs Nos. 16 and 57, that my colleague, Dr. Price Jones, became infected, and 15—17 days later developed a typical attack of Malta Fever.

The question of protective properties may be easily dismissed. In no case up to the present (and some 20 guinea-pigs were employed

for the experiments with the serum obtained in February last) did the subcutaneous injection of even large quantities of the serum, 10 c.c. and 20 c.c., prevent the subsequent infection of the experimental animal by a "standard" dose of *Micrococcus melitensis* injected intracerebrally, or do more than slightly retard the fatal termination. When, however, 0·1 c.c. of serum and a "standard" dose of Micrococci were simultaneously introduced into the cerebral tissue the animal remained unaffected.

Table IV.

Guinea-pig No.	Dose of culture.	Method of inoculation.	Dose of serum.	Method of injection.	Result.
	loops.		c.c.		
140	1	Intracerebrally	5	Subcutaneously	Death in 6 days, *M. melitensis* recovered.
141	2		5	,,	Death in 36 hrs., *M. melitensis* recovered.
142	1		0·1	Intracerebrally	Unaffected.
143	1		—	—	Death in 36 hrs., *M. melitensis* recovered.

The possible possession of therapeutic properties by this serum was next investigated experimentally.

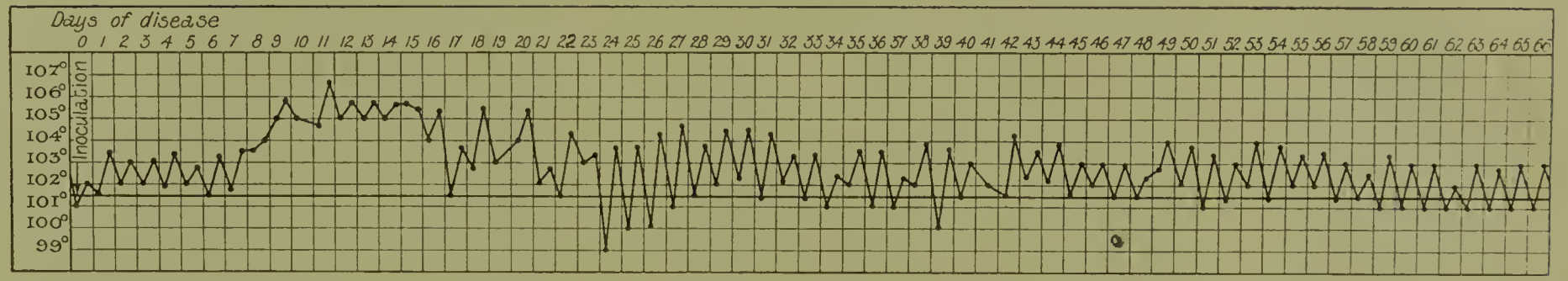
Addendum, October, 1906.

Six healthy monkeys (*Macacus rhesus*) were selected, and each injected subcutaneously with 0·1 of a loopful of cultivation of *Micrococcus melitensis* (grown for 24 hours at 37° C. on an agar slope), emulsified in 1 c.c. normal saline solution. The animals were numbered 1 to 6 inclusive. Eight days later, when signs of successful infection—rise of temperature, appearance of agglutinins in blood, etc.—were apparent, Nos. 1 and 2 were set aside for observation as controls; Nos. 3, 4, and 5 received 3 c.c. horse serum subcutaneously daily for eight days, and No. 6 received 3 c.c. horse serum injected directly into the external saphenous vein daily for a similar period. The result was by no means encouraging, and is well shown in the accompanying series of charts, for while Monkey No. 4 showed a comparatively even temperature and an absence of marked pyrexia that might be attributed to the action of the serum, the charts of the remaining three serum-treated monkeys show no marked differences, so far as concerns the range and duration of pyrexia, from those of the two controls, while throughout the course

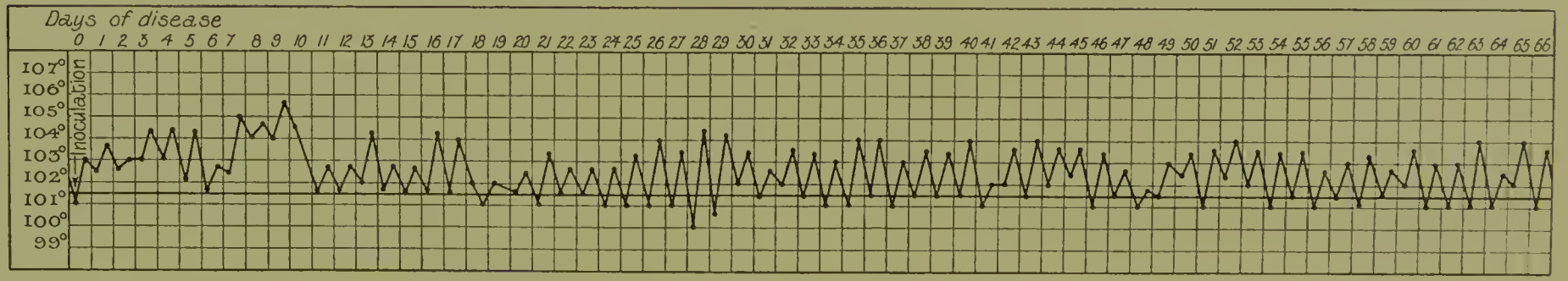
of the experiment simple visual observation of the infected animals was insufficient to enable one to distinguish between those treated with serum and the controls.

The serum was also used in the treatment of one human case, but beyond steadying the pulse and bringing it down from 108 to 96 per minute, a result which might equally well have been achieved by a simple injection of normal saline solution, no further effect could be detected.

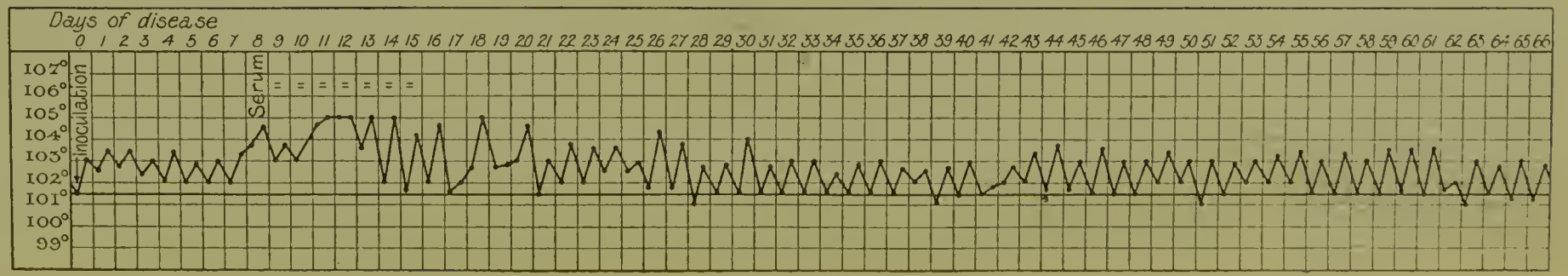
Monkey 1


Days of disease
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 31 32 33 34 35 36 37 38 39 40 41 42 43 44 45 46 47 48 49 50 51 52 53 54 55 56 57 58 59 60 61 62 63 64 65 66
107°
106°
105°
104°
103°
102°
101°
100°
99°
Inoculation

2


Days of disease
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 31 32 33 34 35 36 37 38 39 40 41 42 43 44 45 46 47 48 49 50 51 52 53 54 55 56 57 58 59 60 61 62 63 64 65 66
107°
106°
105°
104°
103°
102°
101°
100°
99°
Inoculation

3


Days of disease
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 31 32 33 34 35 36 37 38 39 40 41 42 43 44 45 46 47 48 49 50 51 52 53 54 55 56 57 58 59 60 61 62 63 64 65 66
107°
106°
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Inoculation
Serum
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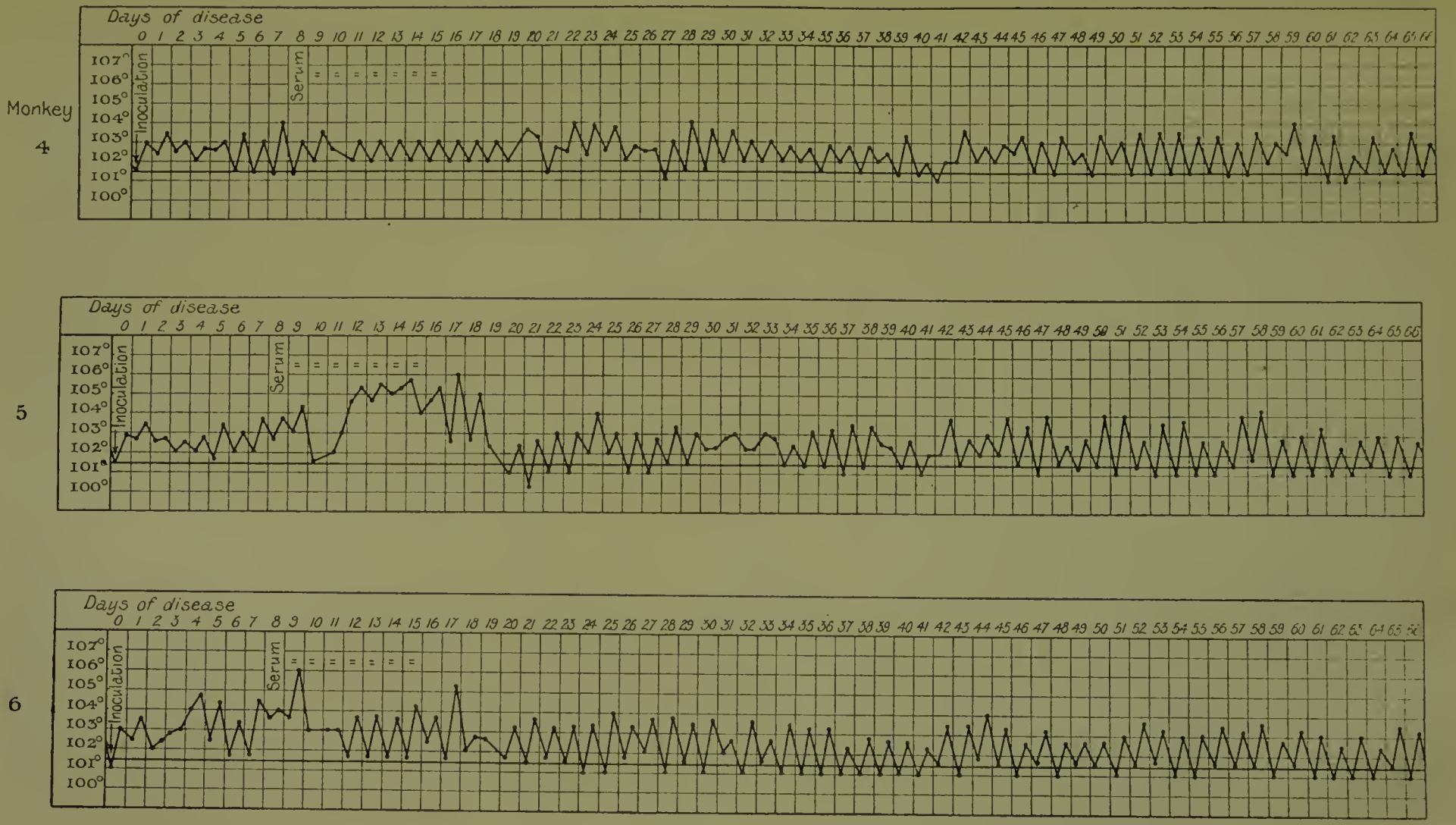
Monkey
4
Days of disease
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 31 32 33 34 35 36 37 38 39 40 41 42 43 44 45 46 47 48 49 50 51 52 53 54 55 56 57 58 59 60 61 62 63 64 65 66
107°
106°
105°
104°
103°
102°
101°
100°
Inoculation
Serum
= = = = = = =
5
Days of disease
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 31 32 33 34 35 36 37 38 39 40 41 42 43 44 45 46 47 48 49 50 51 52 53 54 55 56 57 58 59 60 61 62 63 64 65 66
107°
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104°
103°
102°
101°
100°
Inoculation
Serum
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6
Days of disease
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 31 32 33 34 35 36 37 38 39 40 41 42 43 44 45 46 47 48 49 50 51 52 53 54 55 56 57 58 59 60 61 62 63 64 65 66
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Inoculation
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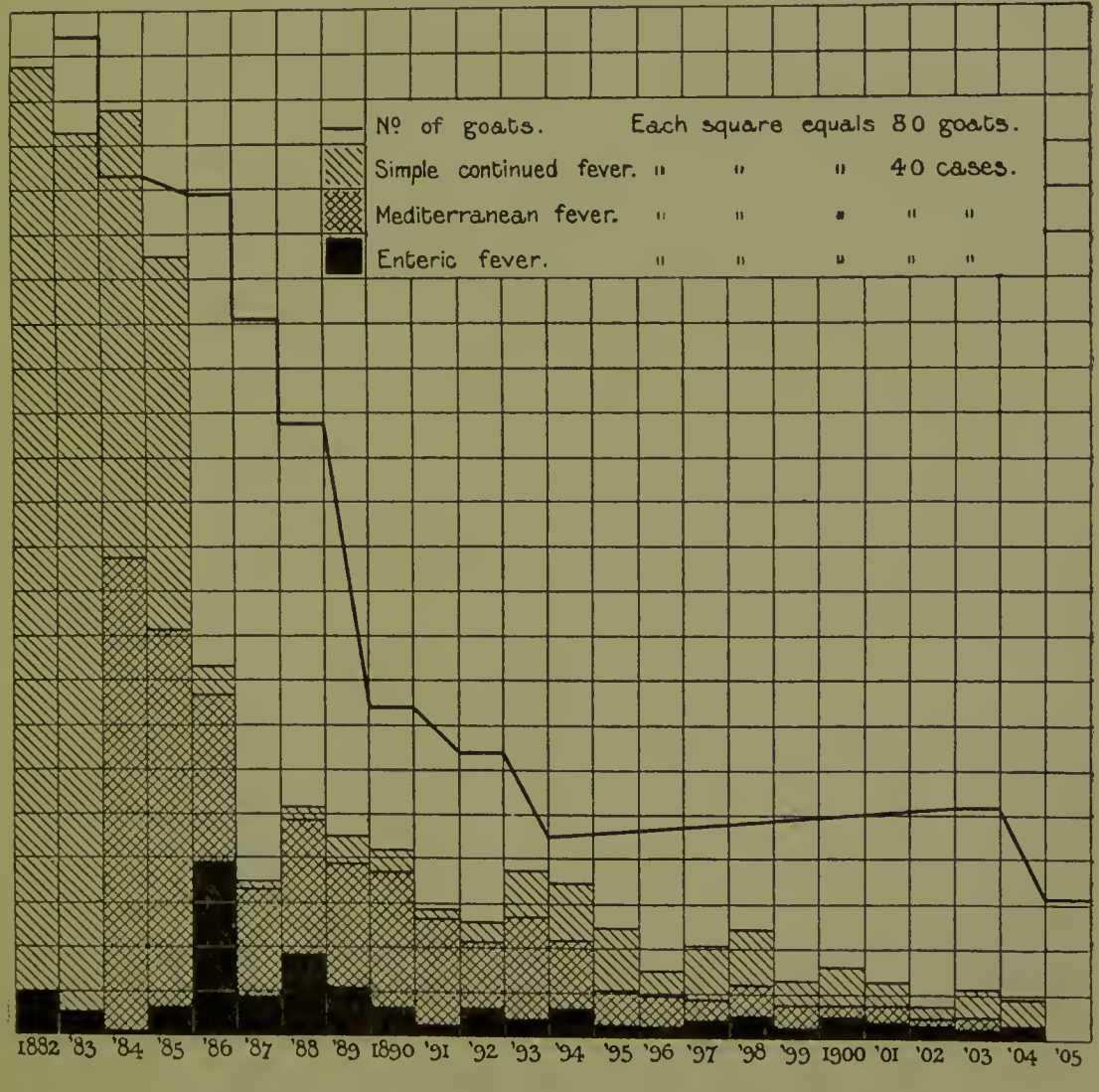
V. MEDITERRANEAN FEVER IN GIBRALTAR.

By Major W. H. Horrocks, R.A.M.C.

Mediterranean Fever, often called "Rock Fever," has existed in Gibraltar for many years. Although the cause of the disease was not known until Bruce isolated the *Micrococcus melitensis* from fatal cases in Malta, medical men practising on the Rock knew of the existence of a fever which was characterised by long duration, low mortality and liability to be followed by rheumatic sequelæ. In the Army Medical Department Report for 1878, it is stated that many of the cases of rheumatism were of an obstinate nature following attacks of Mediterranean Fever, and in the Report for the year 1880 mention is made of 194 cases of rheumatism associated with previous attacks of "Rock Fever." The civil health reports, furnished 25 years ago, do not contain any references to Mediterranean Fever, but in the Report for 1883 there is a statement that the civil population suffered from marked outbreaks of a fever which was not enteric fever and had a very low death-rate. An examination of the returns of the Colonial Hospital shows, however, that the local fever was rarely admitted into that institution. Dr. Turner, the senior colonial surgeon, wrote in a paper on the Rock Fever of Gibraltar, published in 1883, "amongst the indigent poor of the civil community residing in the central districts of the town, I have attended at their homes during 1882 and 1883 over 900 cases of illness; of these 115 have been attributed to continued fever and but two have died. My experience in practice among the upper classes yields similar results. . . . Rheumatism is undoubtedly present in varying degrees of intensity in a large proportion of the cases." There can be no doubt that Mediterranean Fever, as we know it now, was very prevalent both among the civil and military populations some 25 years ago. But as the continued fevers were neither reported to the health officer nor admitted into the Colonial Hospital, except the cases were very severe, it is necessary to examine the military statistics in order to obtain an idea of the real prevalence of the disease. Until the year 1897, when serum diagnosis was practised, the fevers occurring among the military population were returned under the headings, Febricula, Simple Continued Fever and Enteric Fever. The term Remittent Fever was not much used except in connection with cases of fever developing in regiments which had recently arrived from stations where malaria was prevalent.

Unfortunately, the term Simple Continued Fever was used for

febrile attacks of short duration as well as for cases of prolonged fever attended by marked anæmia and complicated by rheumatism, so that in order to make a reliable estimate of the probable number of cases of Mediterranean Fever it has been necessary to examine the Army Medical Reports, the Admission and Discharge Books and the Case Books preserved in the military hospital. A careful examination of these records from the year 1884, shows that if cases of simple continued fever in hospital for 14 days and under are eliminated, a large number of cases remain which are almost certainly Mediterranean Fever. A few cases in hospital for 16 to 20 days are doubtful, but as similar cases observed during the last three or four years gave a serum reaction with the *Micrococcus melitensis*, they have been included in the Mediterranean Fever group. Prior to the year 1884 Admission and Discharge Books are not available, so it is only possible to obtain a general idea of the wave of total fevers on the Rock from the Army Medical Reports. The figures given show that a wave of fever commenced in 1874 and reached a maximum in 1882 when 902 cases of fever were recorded. On the attached chart the total number of cases of continued fever are shown from 1882 to 1905, and from



the year 1884 the proportional parts which enteric fever, Mediterranean Fever and simple continued fever bear to the whole are represented by uniform shading, crossed lines and simple diagonal lines respectively. It will be seen that in the year 1884 there were 833 cases of continued fever, of which 429 were probably Mediterranean Fever. In 1885 there were 697 cases of continued fever, including 341 cases of Mediterranean Fever. In the year 1886, however, there were only 331 cases of continued fever, and of these 158 were returned as enteric fever. The great increase in the number of cases was attributed partly to an infected regiment arriving on the Rock after service in the Egyptian War, and partly to serious sanitary defects in Town Range, Hargreaves, and Buena Vista Barracks. The figures are probably correct, as 26 deaths occurred among the 158 cases of enteric fever. Besides the marked decrease in the number of cases of Mediterranean Fever, the year 1886 is also remarkable for the practical extinction of simple continued fever. In 1887 there was again a considerable fall in the number of cases of Mediterranean Fever, and from that date, with slight oscillations, the curve of Mediterranean Fever gradually declined until it reached zero in 1904.

During the years under review the military population varied between 4,307 in 1886 and 5,031 in 1901, and at the time when the most marked fall in the fever curve occurred the population averaged 4,724. The rapid disappearance of febrile diseases from the Rock, which commenced in 1885, forms a marked contrast to the state of things in Malta during corresponding years. It is plain that some important cause of fever, which has vanished from Gibraltar, has continued to operate in Malta.

Having discovered (1) that the *Micrococcus melitensis* is excreted in urine of men and goats and that animals can be infected by dust contaminated with urine of patients suffering from Mediterranean Fever, and (2) that the *Micrococcus melitensis* is excreted in the milk of infected goats and that the consumption of this milk causes Mediterranean Fever in monkeys, it is evident that both sanitary conditions and possible infection of goats on the Rock must be investigated if the key to the problem is to be found.

Sanitary Conditions of the Rock.

Military Districts.—An examination of the Army Medical Department Reports shows that during the past 30 years there has been a gradual improvement in the sanitary condition of the military districts. In 1872–3 a main drain was made at the New Mole and glazed pipes were used; the joints, however, were made with clay and bedded in mortar. In 1886 the drainage of Town Range, Hargreaves, and Buena Vista Barracks was improved and the waste pipes of ablution rooms were disconnected from sewers. In 1887 the Principal Medical Officer

reported that "the whole system of soil drains requires to be thoroughly overlooked, and nothing short of laying down new soil pipes can possibly meet the urgent requirements under this head . . . the present soil drains of *brick* are defective." In 1888 he stated that "a considerable number of sanitary improvements were effected in the different barracks during the year, principally as regards improvements in drainage, both by structural alterations and ventilation of existing drains." It was customary to make the joints in drainage work with clay up to the year 1890, and most of the drains were of the chair and saddle type. Fresh-air inlets, with mica flaps, for drains were first used about the year 1890. It is evident that up to the year 1888 the military drainage was extremely faulty, and the new work done up to the year 1890 still permitted pollution of the soil owing to the use of clay joints.

Civil Districts.—Up to the year 1865 the civil drains and sewers were constructed with bricks on the box pattern, and the sewage was discharged into the "Bay" on the west side of the Rock. During the years 1865–8 the box drains were removed and earthenware pipes of the chair and saddle type were laid in the north district of the town. A similar change was made in the south district during the years 1870–4. At that time there was no disconnection of house drains from tributary sewers, but in the year 1883 Weaver's siphon trap was installed on civil premises. In 1893 the building bye-laws were passed, and the chief requirements of the Local Government Board model bye-laws were then insisted upon. Fresh-air inlets for house drainage, 4-inch soil pipes placed outside houses, and w.c.'s with separate flushing tanks had to be provided in houses occupied by the civil community. In the year 1896 the new main sewer discharging the town sewage on the east side of the Rock was commenced, and the work was completed in 1898.

As the curve representing febrile diseases amongst the military population steadily rose from 1874 to 1884, it is unlikely that the improved civil drainage was the principal cause of the reduction of fever that suddenly commenced in 1885. The disconnection of civil premises from tributary sewers may have had an influence in diminishing febrile attacks among the civil population. Up to and including the year 1883 the civil Health Reports contain several references to outbreaks of fever, not enteric, amongst the civil population, but in the two years following the house disconnection febrile diseases are stated to be not so prevalent as formerly. There are, however, no figures in the Health Reports to support these general statements.

Examination of Goats.

Twenty years ago goats were allowed to graze on the upper portions of the western side of the Rock, and for this purpose passes were granted to goat-keepers by the Royal Engineers. On consulting the records in the office of the War Office Lands, it appears that in the year 1883 passes for 1,795 goats were granted. During the year 252 goats were sold; consequently, in 1884, there were 1,543 goats on the Rock. In 1886 the Royal Engineer records showed passes granted for 1,512 goats. In 1887 only 1,285 passes were given, and these were reduced to 1,104 in 1888. In 1890 the passes were reduced to 590, and in 1892 some 80 of these were cancelled. In 1893 the War Office took possession of the ground below Ince's Farm and 150 goats kept there at the time were sold. From 1894 to 1902 the number of goats appears to have changed very little. An examination made by Sanitary Inspector Balestreno at the end of 1903 showed 413 goats to be present. In 1904 the passes were reduced to 210, and when I commenced the examination of goats in 1905 I found 254 goats distributed on the various parts of the Rock.

It might be urged that though passes for grazing were withdrawn, the goats were still kept and fed in the goat-sheds. This, however, was not the case, as from information supplied by former goat-keepers, who no longer follow the trade, I have ascertained that from 1883 to 1893 about 1,100 goats were sold. As many Maltese goat-keepers who used to keep goats have left the Rock, it is not possible to trace the fate of all the goats present in Gibraltar in 1883. Still, as the Maltese now following the goat trade assure me that goats were not kept in any numbers when passes for grazing could not be obtained, and as the War Office took over the land upon which many of the old goat-sheds were built, I think the figures given above may be taken as representing fairly accurately the number of goats on the Rock during the years mentioned.

It is interesting to note that in 1883 practically all the goats on the Rock were Maltese, and at that time regular shipments of goats from Malta to Gibraltar took place. *Pari passu* with the withdrawal of grazing passes and the increase in the cost of shipment, the importation of goats from Malta on a large scale ceased, and goat-keepers replaced their stock partly by importation of Spanish goats and partly by breeding. In this way three classes of goats were obtained, viz.: (1) Spanish goats; (2) Maltese goats, descendants of the goats originally brought from Malta; (3) "mixed" goats, obtained mainly by breeding from Maltese fathers and Spanish mothers.

Infection of Goats Existing on the Rock in 1905.—Specimens of blood were taken from 254 goats found on various parts of the Rock and tested in the usual manner with a recent culture of the *Micrococcus*

melitensis, dilutions of serum from 1 in 10 to 1 in 100 being made. The results obtained are given in the attached table, which shows that 14 per cent. of the goats gave a reaction with the *Micrococcus melitensis*. There appears to be very little difference between the infection (about 15 per cent.) of the Maltese and "mixed" breeds. Of the Spanish goats, however, only 11 per cent. seem to be infected. Samples of milk were taken from all the goats, except those that were pregnant, and tested for agglutination. The milk from Nos. 3 and 4, both Maltese goats, in Goat-shed No. 1, Engineer Road, caused immediate clumping of a rich emulsion of the *Micrococcus melitensis*. None of the other samples of milk, whether from infected or non-infected goats, gave any signs of a reaction. Ten cubic centimetres of each sample of milk were then centrifugalised and the deposit plated on glucose-nutrose-litmus-agar. Numerous colonies of the *Micrococcus melitensis* were found in the plates made with the milk from the Maltese Goat No. 4, Engineer Road. It was expected that the specific organism would also be found in the milk of Goat No. 3, Engineer Road, but though it was repeatedly examined, no signs of the *Micrococcus melitensis* could be detected. Five cubic centimetres of blood were taken from Goat No. 4 on three different occasions and planted out in broth, but no growth occurred. An examination of the milk was made at frequent intervals during the next three months, but the *Micrococcus melitensis* never appeared again; consequently, it would seem that Goat No. 4 had not been recently infected.

It will be noticed that the dilutions of the sera, which reacted with the *Micrococcus melitensis*, are mostly low, only two sera reacting in a dilution of 1 in 100 and six in a dilution of 1 in 50. These reactions also suggest that many of the goats are probably in a late stage of the disease. A re-examination of the goats made six months later proved this to be the case, as the sera, which formerly reacted in dilutions of 1 in 100 and 1 in 50, then only reacted in dilutions of 1 in 20 and 1 in 10, and many of the sera reacting in dilutions of 1 in 20 and 1 in 10 gave no reactions at all.

Further evidence of infection, not of recent date, was also obtained by examining the cows in Mr. Patron's dairy. These cows are kept under exceptionally good sanitary conditions, and though they are stall-fed in Gibraltar, a constant interchange with cows kept at the farm in Spain is kept up. When I commenced the examination there were 12 cows in the dairy and 49 cows at the farm. The serum of one of the cows (Huelfanita) in the dairy, when diluted 1 in 100, caused instantaneous clumping of the *Micrococcus melitensis*. This cow had recently calved, and was in a bad state of health owing to a retained placenta. The first secretion of milk, diluted 1 in 100, was also found to agglutinate the *Micrococcus melitensis*. During the

next 14 days 30 c.c. of the milk were centrifugalised daily and the deposit plated. The *Micrococcus melitensis* was never recovered. A week later the cow died, and at the *post-mortem* examination the spleen was found small and firm in consistence; the glands also were small and fibrous in texture. Cultures were made from the spleen, glands, liver, and kidneys, but no signs of the *Micrococcus melitensis* appeared. From the appearances found at the *post-mortem* examination it is certain that the cow, Huelfanita, had not been recently infected. The cows at the farm were next examined, and the serum taken from one of them, when diluted 1 in 20, was found to react with the *Micrococcus melitensis*.

Mode of Infection of the Goats on the Rock.—It appears probable that infected goats were imported amongst the herds brought from Malta, but the disease now existing cannot have a direct Maltese origin, as very few goats belonging to the imported stock now remain, and none of these are infected. It might be suggested that many of the goats now on the Rock are not really infected, and that the serum reactions given by the descendants of the old stock are due to agglutinations transmitted *in utero* from infected parents. In the last report of the Mediterranean Fever Commission it was shown that agglutinins are sometimes transmitted from an infected mother to the kid. The transmission of agglutinins was also noticed in the case of several kids born of infected mothers in Gibraltar, and the serum of the calf of the cow, Huelfanita, mentioned above, was also found to agglutinate the *Micrococcus melitensis*. But as these agglutinins did not persist for more than six weeks, and the serum reactions given in the table were found little changed at the end of three months, it is not likely that they were caused by agglutinins transmitted from infected mothers, and the goats must be considered really infected.

The transmission of the *Micrococcus melitensis* from mother to kid could not be demonstrated in Malta, and in Gibraltar two apparently infected kids were killed immediately after birth, and cultures were made from the organs. Though more than 80 cultures were made not a sign of the *Micrococcus melitensis* could be discovered. It is evident, therefore, that the disease now existing amongst the goats must have been acquired on the Rock. In previous reports I have shown that the *Micrococcus melitensis* is excreted in the urine of goats, and that healthy goats can be infected by food contaminated with urine containing the specific microbe. The evidence obtained from a study of the goats in the shed at Palace Gully Steps strongly supports the idea that this is a mode of infection now operating amongst the goats on the Rock. The goats were first examined at the end of October, 1905, and five goats were found to be infected. At the end of March, 1906, the herd was re-examined, when five other goats, which were found perfectly healthy at the first examination,

showed a blood reaction, and from the milk of one of these goats the *Micrococcus melitensis* was isolated. The goats became infected during the winter months, when biting flies and mosquitoes were not to be found.

I noticed, however, that the coats of the goats were infested with pediculi, and as many of these were full of blood, I thought they might possibly act as a means of conveying infection. Accordingly, specimens full of blood were taken from infected goats and thoroughly washed with water, the thorax and abdomen were then opened with a sharp curved bistoury, and the blood and viscera were transferred to a sterile glass slide. A little sterile salt solution was then added, and the blood and viscera having been thoroughly mixed with it, the mixture was drawn up in a capillary pipette and then plated on glucose-litmus-nutrose-agar. More than 150 pediculi were examined in this manner, but no signs of the *Micrococcus melitenis* were discovered.

The goats in the shed at Palace Gully Steps, during the winter months, were under the same conditions as the contact experiments, related in the Fourth Report, in which diseased and healthy monkeys were allowed full contact, mosquitoes and flies being excluded by mosquito netting. Under the conditions described, healthy monkeys became infected by associating with diseased monkeys, and the infection could only be attributed to micrococci contained in the urine of the infected monkeys. Other sources of infection being excluded, it appears certain that goats during the winter months may become infected in this manner.

During the summer months it is possible, as I stated in the Fourth Report, that goats and cows may be infected by mosquitoes which have fed on infected men and animals; but of this mode of infection I could not obtain any evidence. In one shed, during the summer months, a healthy herd of goats was only separated by a wooden partition from diseased goats, but no infection of the healthy goats occurred, though *Culex pipiens* and *Stegomyia fasciata*, which have been shown to act as carriers of the *Micrococcus melitensis*, are found abundantly on the Rock. This result I attributed to the facts that there were no cases of Mediterranean Fever amongst human beings in the vicinity of the goat-sheds, and that the infected goats were in a chronic state of the disease, no evidence of the presence of the *Micrococcus melitensis* in their blood or milk being obtainable at the time.

The evidence of disease amongst the Spanish goats in Gibraltar was very interesting, and raised the question whether goats living on the hills and in the towns in Spain are infected. The Spanish goats living on the Rock were always associated with goats of the Maltese and "mixed" breeds, so they might easily have become infected in the manner just described. But as goat-keepers are now introducing

Situation of goat-shed.	Number of goats.				Number of infected goats.				Infected goats.					
	Spanish (S.).	Maltese (✝).	Mixed (M.).	Total.	Spanish.	Maltese.	Mixed.	Total.	Age.	Place of birth.	Dilution of serum reacting with *M. melitensis*.			
									years.		1/10.	1/20.	1/50.	1/100.
Palace Gully Steps...	13	19	5	37	0	3	2	5	(1) M. 2.....	Gibraltar	+	+	+	+
									(2) M. 2.....	,,	+	0	0	0
									(3) ✝ 1.....	,,	+	+	0	0
									(4) ✝ 4.....	,,	+	+	+	0
									(5) ✝ 6.....	,,	+	0	0	0
Lime Kiln Gully.....	1	1	13	15	0	0	5	5	(1) M. 6.....	Gibraltar	+	+	0	0
									(2) M. 2.....	,,	+	+	0	0
									(3) M. 2.....	,,	+	0	0	0
									(4) M. $1\frac{6}{12}$.....	,,	+	0	0	0
									(5) M. 4.....	,,	+	0	0	0
Engineer Road, Goat-shed No. 1 (F. F.)	1	29	1	31	0	5	1	6	(1) ✝ 3.....	Gibraltar	+	0	0	0
									(2) ✝ 5.....	,,	+	+	+	0
									(3) ✝ 2.....	,,	+	+	+	+
									*(4) ✝ 3.....	,,	±	0	0	0
									(5) ✝ 2.....	,,	+	0	0	0
									(6) M. 3.....	,,	+	0	0	0
Engineer Road, Goat-shed No. 2 (T. D.)	10	13	0	23	0	3	0	3	(1) ✝ 3.....	Gibraltar	+	0	0	0
									(2) ✝ 2.....	,,	+	0	0	0
									(3) ✝ 2.....	,,	+	0	0	0
Engineer Road, Goat-shed No. 3 (T. V.)	8	0	29	37	0	0	0	0	—	—	—	—	—	—

Engineer Road, Goat-shed No. 4 (A. C.)	8	1	10	19	3	0	1	4	(1) S. (on the Rock $2\frac{3}{12}$ years)	Spain	+	0	0	0
									(2) S. (do., 3 years)	,,	+	0	0	0
									(3) S. (do., 4 ,,)	,,	+	0	0	0
									(4) M., age 4 ,,	Gibraltar	+	0	0	0
Naval Hospital Hill...	5	8	15	28	1	1	3	5	(1) S. (on the Rock 6 years)	Spain	+	+	0	0
									(2) M., age 4 years	Gibraltar	+	0	0	0
									(3) M., ,, $1\frac{2}{12}$,,	,,	+	0	0	0
									(4) M., ,, 4 ,,	,,	+	0	0	0
									(5) ✝, ,, 5 ,,	,,	+	0	0	0
Rosia.....	10	8	21	39	3	2	3	8	(1) S. (on the Rock 2 years)	Spain	+	0	0	0
									(2) S. (do., 6 years)	,,	+	+	0	0
									(3) S. (do., 4 ,,)	,,	+	+	+	0
									(4) ✝, age 3 years	Gibraltar	+	0	0	0
									(5) ✝, ,, $2\frac{6}{12}$,,	,,	+	+	+	0
									(6) M., ,, 3 ,,	,,	+	0	0	0
									(7) M., ,, $1\frac{8}{12}$,,	,,	+	0	0	0
									(8) M., ,, $1\frac{8}{12}$,,	,,	+	0	0	0
Catlan Bay	0	9	7	16	0	0	0	0	—	—	—	—	—	—
Almeda.....	6	2	1	9	0	0	0	0	—	—	—	—	—	—
Total	62	90	102	254	7	14	15	36	—	—	—	—	—	—

* *Note.*—The *M. melitensis* was recovered from the milk of No. (4).

Spanish goats to replace the Maltese, it was of great importance to find out whether those entering the Fortress showed any signs of disease. With this object in view, I examined a herd of 50 goats living in sheds on the hills 10 miles from Gibraltar. The goats were all pure Spanish breed and, so far as I could ascertain, had never been associated with Maltese goats or lived in the neighbouring towns.

Specimens of blood were taken from the goats and examined in the usual manner, but no serum reactions with the *Micrococcus melitensis* were obtained; all the goats appeared perfectly healthy. Different results, however, were obtained when I examined goats which had lived in the Spanish towns of Malaga and Linea. The goat-keepers informed me that the goats they were then importing came from Malaga, and I was led to suspect that these goats might be infected, as I had learnt that when the great exodus of goats from the Rock occurred, many of them were taken to Linea and Malaga as well as to Oran, Algiers, Tangier, and other towns on the African coast. The appearance of some of the goats arriving from Malaga also suggested an admixture of Maltese and Spanish breeds.

Just after I had examined the Spanish country goats, a small herd of 16 goats was brought from Malaga, and, after living in Linea for three months, was allowed to enter the Fortress. I immediately visited the herd and found that while 10 were obviously pure Spanish goats, six showed distinct evidence of a Maltese strain. Samples of blood were taken and the sera were tested in the usual manner. Two of the Spanish goats gave reactions when the sera were diluted 1 in 10, and two of the mixed breed also gave a reaction, one with the serum diluted 1 in 50 and the other with the serum diluted 1 in 10. Samples of milk were then obtained, and 10 c.c. of each having been centrifugalised, the deposit was plated in the usual manner. Each sample of milk was also tested as to its agglutinating action on the *Micrococcus melitensis*. The results were uniformly negative; no signs of the specific microbe were observed in the plates and the milk had no agglutinating power. The goats in this small herd were previously in much the same condition as many of the goats now living on the Rock, notably those found in the shed at Palace Gully Steps.

At the first examination the milk of the infected goats in the shed did not contain the specific microbe, and had no agglutinating action, and yet other healthy goats associating with them became infected. Consequently, the admission of goats from towns in Spain cannot be considered free from danger, and in the interests of the public health it is plain that all goats brought to Gibraltar should be quarantined until examinations of the blood have shown them to be free from infection.

Conclusion.

It appears probable that the rapid disappearance of Mediterranean Fever from Gibraltar, which commenced in 1885, was intimately associated with the exodus of infected goats from the Rock. Improved sanitary conditions, especially the disconnection of waste-pipes and house-drains from sewers, may have played a part in causing the decrease of fever, but as the same sanitary improvements have been carried out in Malta without any corresponding decline of Mediterranean Fever, it is fair to assume that their effect was insignificant compared with that produced by the removal of infected goats.

VI. BIBLIOGRAPHY OF MEDITERRANEAN FEVER. 1897 to 1907.

Compiled by J. W. H. Eyre, M.D., Referee for Volume *R* (Bacteriology) of the International Catalogue of Scientific Literature.

The late Surgeon-Captain Louis Hughes included a comprehensive bibliography of Mediterranean Fever, ranging in point of time from the *Epidemics* of Hippocrates (460 to 357 B.C.) to the date of publication, in his own monograph on the subject, which was entitled "Undulant, Malta, or Mediterranean Fever" (Macmillan and Co., 1897). In this work the bibliography occupies the position of an appendix to Chapter I (pp. 29 to 34).

In the compilation of his list—a work in which he had the advantage of Professor Zammit's assistance—Hughes searched through many rare and some unique volumes, both in manuscript and in type, lodged in the Bibliotheca at Valetta, and so secured many valuable references not elsewhere obtainable.

The present list is a continuation of Hughes' bibliography, and embraces the period from the year 1897 to the present date—January, 1907. In it the arrangement of the papers bearing on the subject, which Hughes adopted, viz., grouping together such as appeared in the same year, has been followed, so that continuity is secured; but, as during the past few years the papers on Mediterranean Fever have, in the main, been written by a few observers, the references are also arranged in a separate section in alphabetical order under their respective author's names, in such a manner as to admit of their utilisation for the purposes of a card index, and corrections or additions to this list will be gratefully received by its compiler. The details relating to each reference are arranged according to the plan adopted by the International Catalogue of Scientific Literature,* to the Central Bureau of which—through its Director, Dr. Forster Morley—and particularly to the Regional Bureaus of Germany, Greece, and Italy, the writer wishes to express his thanks for the valuable assistance he has received in the preparation of this list; as well as to the many correspondents whose cordial help has been highly appreciated.

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* Such was the original intention of the author of the Bibliography, but it has not been considered advisable to follow the plan.—[Sec. R.S.]

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